Investigating the effects and potential impacts of lionfish (*Pterois* spp.) in low salinity habitats.

Submitted by Rebekah Trehern to the University of Exeter for the degree of Masters by Research in Biological Sciences in April 2019.

Supervisors: Dr Lucy Hawkes and Dr Travis Van Leeuwen

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Abstract

In this thesis, I use physiological and behavioural experiments in a laboratory environment to assess the impacts and potential effects of the invasive lionfish (*Pterois* spp. (Wilcox et al. 2018)) in a mangrove ecosystem. **Chapter 1** of the thesis is a general introduction to the main theme of the thesis "lionfish in mangroves". In this chapter, I present an introduction to invasive species, a general background about the introduction of lionfish in the Western Atlantic and wider Caribbean, the importance of mangrove ecosystems, and then end with the recent discovery of lionfish in mangrove ecosystems. **Chapters 2** and **3** are the data chapters of the thesis. In **Chapter 2**, lionfish were reared in aquaria in the Bahamas under differing salinity treatments (37 ppt (typical reef ecosystem salinity), 20 ppt (typical mangrove ecosystem salinity) and 10 ppt (typical mangrove ecosystem salinity during low tide)) for 84 days. This experiment investigated the effect of acute hyposalinity on growth rate, metabolic rate (standard metabolic rate (SMR), maximal metabolic rate (MMR) and aerobic scope (AS)), maximum food consumption, feeding rates and specific dynamic action (SDA_{Max}, SDA_{Scope}, SDA_{Total}, $SDA_{Duration}$). Most lionfish survived in hyposaline conditions for 84 days until the conclusion of the experiment, however, various aspects of their physiology were effected. Lionfish feeding and aerobic capacity (MMR and AS) were reduced, whereas digestive duration ($SDA_{Duration}$) increased at the lowest salinity. Therefore, I suggest: 1) given the ability of lionfish to tolerate low salinity, updated range expansion models should incorporate salinity data to improve accuracy of predicted range expansion for lionfish, and 2) the invasion of lionfish in mangrove habitats with low salinity, although a serious concern, will unlikely lead to the same level of population increase, habitat destruction and decline in native fish populations as observed for some

Caribbean coral reef habitats. In **Chapter 3**, I explore the potential impact of lionfish on a native fish species, which typically occupies a similar trophic niche as lionfish in an invaded mangrove ecosystem, the Schoolmaster snapper (*Lutjanus apodus*). Using a habitat competition experiment in the laboratory at two salinity treatments (10 ppt (typical mangrove ecosystem salinity during low tide) and 37 ppt (typical reef ecosystem salinity)), I investigated the behavioural interactions between invasive lionfish and native Schoolmaster snapper in a simulated mangrove ecosystem. Schoolmaster snapper showed, on average, a 53% reduction in shelter use when in the presence of lionfish. Results suggest that lionfish have the ability to displace snapper from shelter and are therefore likely to increase the vulnerability of native mangrove fish species to predation by other mangrove meso-predators. Using a behavioural ethogram, I further identified key behaviours displayed by both fish species during a period in which lionfish and snapper both had access to shelter. However, salinity had no effect on the occurrence and outcome of these behaviours, despite my findings in Chapter 2. **Chapter 4** is the general discussion of the thesis where I draw on previous research, as well as research from this study to place in context the novel threat of the lionfish invasion in mangroves.

Overall, this thesis investigates the invasiveness of lionfish in a mangrove ecosystem by demonstrating their ability to tolerate and function in low salinity water for extended periods.

Table of Contents

Table of Contents....4
List of Tables....6
List of Figures....7
Author's Declaration....10
Acknowledgements....11
Chapter 1: General Introduction....13
An introduction to invasive species....13
Lionfish and their invasion....14
The importance of mangrove habitats....16
Lionfish in mangroves....17
Thesis research....17
Chapter 2: Tolerances of a marine invader: bioenergetic responses of lionfish (*Pterois spp.*) to an environment of lowered salinity....22
Introduction....22
Methods....27
Animal capture and transport....27
Lionfish husbandry and feeding....28
Salinity treatments....30
Measuring metabolic rates....30
Measuring maximal metabolic rate (MMR)....32
Standard metabolic rate (SMR) and determination of aerobic scope (AS) 33
Specific dynamic action (SDA)....33
Calculations and statistical analyses....34
Ethics....35
Results....35
Growth, average prey consumption time and maximum food consumption....35
Standard metabolic rate, maximal metabolic rate and aerobic scope....36
Specific dynamic action....36
Mortalities....36
Discussion....37
Figures and Tables....43
Chapter 3: The lion, the fish, and the mangrove: interactions between Schoolmaster snapper and invasive lionfish in a simulated mangrove ecosystem....48
Introduction....48
Methods....53

Animal capture and transport 53
Animal rearing, husbandry and feeding 53
Salinity treatments 54
Behavioural interactions 54
Video analysis 55
Behavioural ethogram 56
Calculations and statistical analyses 56
Ethics 56
Results 57
Discussion 58
Figures and Tables 64
Chapter 4: General Discussion 71
Bibliography 74

List of Tables

Table 1.1 A comprehensive list of publications identifying lionfish in mangroves and other low salinity habitats including: location, methodology and main conclusions……..……..……...19

Table 2.1 Experiment values for lionfish acclimated to three salinity treatments (low / 10 ppt and 20 ppt and control / 37 ppt). Values represent the mean ± SE; (a) indicates metrics that were statistically significant between 20 ppt and control, (b) indicates metrics that were statistically significant between 10 ppt and 37ppt, and (c) indicates metrics that were statistically significant between 20 ppt and 10 ppt, all significance is at 5%.

NB: Due to unexpected mortalities during the high food portion of the growth experiment only data from the 10 ppt and 37 ppt treatments were compared……..44

Table 3.1 A comprehensive description of behaviours included in the behavioural ethogram and defined from previous research on aggressive behaviours displayed by lionfish and passive behaviours displayed by snapper. (a) denotes fear behaviours displayed by the snapper, (b) denotes inquisitive behaviours displayed by the snapper towards the lionfish and (c) denotes aggressive behaviours displayed by the lionfish towards the snapper 67

Table 3.2 Experiment values for behaviours displayed by lionfish and snapper during interaction trials and when acclimated to two salinity treatments (low / 10 ppt and control / 37 ppt). Values represent the mean ± SE……..68

List of Figures

Figure 1.1 Map of the native and invasive ranges of *Pterois* spp. Sourced from https://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=963……...21

Figure 2.1 A schematic of the intermittent-flow respirometry set-up used to measure oxygen consumption, and determine the metabolic phenotype and digestive efficiency of individual lionfish. Solid lines indicate flow of water; dotted lines indicate flow of information……..……..……43

Figure 2.2 The relationship between salinity (low / 10 ppt and 20 ppt and control / 37 ppt) and mean growth rate residual corrected for body mass (rGrowth; % body length / day) for; **(A)** lionfish fed a low food ration for 24 days and **(B)** lionfish fed on a high food ration for 14 days. Dark points show the mean growth for each treatment, error bars represent ± SE and faded points show all data collected. Residuals were calculated from the regression equation of growth (% length / day) against body mass (g) for all fish.
NB: Due to unexpected mortalities in the 20 ppt treatment during the high food ration experiment only fish from the 10 ppt and 37 ppt treatments are compared **(B)** ……..……..……..……..…….46

Figure 2.3 The relationship between salinity (low / 10 ppt and 20 ppt and control / 37 ppt) and metabolic rate for; **(A)** maximum metabolic rate (residual corrected for body mass (MMR; mg O_2 / h)) for lionfish (n = 40), **(B)** aerobic scope (residual corrected for body mass (rAS; mg O_2 / h)) for lionfish (n = 40), **(C)** maximum food

consumption (g) for lionfish (n = 50) and **(D)** the duration of the specific dynamic action response ($SDA_{Duration}$; in hrs) for lionfish (n = 36).
Residuals for MMR and AS were calculated from the regression equation of the whole values (mgO_2 / hr) against body mass (g) for all fish. Dark points represent the mean value for each treatment, error bars represent ± SE and faded points represent all data collected 47

Figure 3.1 A schematic showing the three experimental stages filmed for each behavioural trial; **(A)** an isolation period in which only the Schoolmaster snapper had access to the shelter, **(B)** an interaction period in which the Schoolmaster snapper and lionfish both had access to the shelter and **(C)** an isolation period in which only the lionfish had access to the shelter. All fish were given 24 hours to acclimatise to the experimental arena, with each experimental stage filmed for 90 minutes. Each trial was conducted in the relevant salinity in which the subjects had been acclimated for 1 month 64

Figure 3.2 Top down photograph of the experimental arena used during the interaction stage of the experiment. The arena was split into a grid system to allow for positions of fish to be determined. The lionfish is shown by the solid circle, the Schoolmaster snapper by the dashed circle and the mangrove (shelter) by the square.....65

Figure 3.3 The relationship between mean time spent in the cover of a mangrove (min.) and salinity (low / 10 ppt and control / 37 ppt) for Schoolmaster snapper (black symbols) and lionfish (white symbols) for both an isolation period (circles), where the snapper or lionfish only had access to the mangrove shelter and an

interaction period (squares), where the snapper and lionfish both had access to the mangrove shelter. Error bars represent 95% CI.
NB: The significant decrease in shelter use of the Schoolmaster snapper in both salinity treatments (10 ppt and 37 ppt) when competing for shelter with lionfish. ……..……..……..……..……66

Figure 3.4 The relationship between average count of each behaviour and salinity (low / 10 ppt and control / 37 ppt) during the interaction period, when snapper and lionfish both had access to the mangrove shelter (n = 12). Error bars represent ± SE.....69

Figure 3.5 A schematic diagram showing; **(A)** the current predicted food chain within a mangrove ecosystem, before the introduction of lionfish and **(B)** the potential trophic cascades as a result of the introduction of lionfish into a mangrove ecosystem. The introduction of lionfish, as suggested by the present study, could cause snapper to move into the open and cause increased predation. In turn this could cause a reduction in predator numbers and a decrease in fishing trade due to a decrease in snapper population size from increased predation. The dotted lines represent a predicted change in fishing trade as there is now an increasing market for lionfish and also that lionfish may begin to prey on small snapper if prey supplies become limited 70

Author's Declaration

This work was carried out in collaboration with the Cape Eleuthera Institute, The Bahamas. I coordinated, supervised and conducted fieldwork, which included; subject collection, animal husbandry, respirometry and behaviour set-up and trials, and data collection. I also co-lead and coordinated an Island School Research Class based upon my research, in which students assisted with the collection of data for Chapter 3. Following this, I remotely carried out all subsequent data analysis, thesis writing, and formatting. Drs. Lucy Hawkes and Travis Van Leeuwen provided guidance and comments throughout the project. Professor Iain McGaw also aided during the data collection, experimental set up and analysis, and additional comments on the manuscript for Chapter 2.

All material in this thesis has been referenced if it is not my own work. For all images which I do not own, the link to access these images has been provided.

The project was designed and based upon work conducted by Dr Travis Van Leeuwen at the Cape Eleuthera Institute.

Acknowledgements

There are many people that I would like to thank for their support and guidance throughout this project. Firstly, I would like to thank my supervisors, Drs. Travis Van Leeuwen and Lucy Hawkes, for giving me this amazing opportunity and for their continued support. A special thank you to Travis for the constructive edits you have provided and for pushing me to continue, even when I began to doubt myself.

I would also like the thank the Research Technicians, staff and interns at the Cape Eleuthera Institute, Spring 2017 for their contribution to data collection. Specifically, Logan Zeinert, Cameron Raguse and Jodie Ball for boat support and Team Travis; Hannah Hauptman, Aneri Garg and Bill Bigelow for all their help, without whom, this research would never have been possible. I would also like to thank the Office Girls; Jessica Rudd, Izzy Lake and Tanya Scwank for their fun, love and support during the gruelling 6 months of research.

Specifically, for Chapter 3, I would like to thank the students of the Island School Spring 2017 Lionfish in Mangroves research class, Andrew Treater, Aukai Elkaslasy, Nina Sanchez, Ainsley Green, Nevin Ketchum and David Mohammed for their hard work, commitment and contribution to the research during the semester.

A very special thank you to my Mum, Dad and sister, for always being on my side and supporting every crazy adventure I decide to take. Big thank you to Alice Rosen, Sophie Hedges, Ellen Whitby, Emma Dwan and Daniel Terriaca for believing in me when I didn't believe in myself, for being the best friends anyone could ask for. Thank you to Aidan Seaton for his love, support and confidence in

me and for continually asking when I would finish. Thank you for helping right at the end, when I needed it the most.

And finally, for my Grandad and my Dan who passed away before they could see my masters finished. They always believed in me; this is for both of you.

Chapter 1: General Introduction

An introduction to invasive species

The term 'invasive species' within the scientific community has been defined as a widespread, non-native species that has adverse effects on the new ecosystem (Davis et al. 2000; Mack et al. 2000). Some non-native species can migrate into habitats without causing significant changes to the ecosystem, whereas others can have devastating consequences (Bax et al. 2003). For example, the Cane toad (*Bufo marinus*) invasion in Australia has been one of the most damaging, but best studied invasions of all time, causing a loss of many native fauna (Sabath et al. 1981; Freeland & Martin 1985). In certain parts of Western Australia, the toads were found to have caused a 50% reduction in some water monitor (*Varanus salvator*) populations within five years (Doody et al. 2014).

From land to sea, invasive species can have detrimental effects, not just upon the habitat and native species, but also human health and industry (Vitousek et al. 1996; Ruiz et al. 2015). For example, invasive species that are vectors for pathogens, such as the mosquito (*Family: Culicidae*), may alter disease transmission dynamics and create new human health issues (Juliano & Lounibos 2005). Introductions can be either intentional or accidental, with the major facilitators of global marine invasions being international shipping through ballast and/or fouling (69%), followed by aquaculture (41%), canal construction (17%) and the aquarium trade (6%) (Molnar et al. 2008). Over 84% of marine environments have now reported the presence of non-native species (Molnar et al. 2008). Therefore, the expansion of non-native species needs to be closely monitored, as they present both a regional and global threat (Bax et al. 2003).

Mitigation is also a vital reason for increased research into invasive species. Following the increase of research investigating the negative impacts of lionfish in the invasive range, government and community initiatives are being pushed to mitigate the potential impacts and the species. Many communities now encourage the collection of lionfish within competitive fishing and through the commercial fishing trade (Gallagher 2013; Huth et al. 2016; Chapman et al. 2016). This work further identifies how multi-sector collaborations are key to effective lionfish management.

Lionfish and their invasion

Marine fish introductions are still considered rare, with the environmental effects often under-studied (Baltz 1991). This is largely due to the difficulties associated with conducting invasion impact studies in marine environments (Bax et al. 2003). However, the invasion of Western Atlantic and Caribbean coral reefs by the Indo-Pacific lionfish (*Pterois* spp.*)* has been considered as an environmental challenge that could have substantial impacts on the conservation of biological diversity (Sutherland et al. 2010). Since it's identification as a threat in 2009, the lionfish has colonised all sub-regions of the Caribbean Sea (Sutherland et al. 2010). In terms of conservation biology, the invasion does rank below new technology (23%), climate change (18%) and societal change (14%) (Sutherland et al. 2010). However, the occurring impact of lionfish on invaded ecosystems cannot be overlooked (Sutherland et al. 2010).

The first confirmed sighting of lionfish in the Western Atlantic, occurred in Florida, USA in 1985 (Whitfield & Hare 2003; Morris & Akins 2009). In 1995, it was suggested that six more lionfish were accidentally released from an aquarium into Biscayne Bay, Florida during Hurricane Andrew (Courtenay 1995). However, this claim was retracted in 2010 due to a lack of evidence and apparent

misinformation (Ingeman et al. 2017). From 1992 to 2000 there were no recorded sightings of lionfish, until eight more were observed off the coast of Florida, South Carolina and North Carolina, USA, in 2002 (Schofield 2009). Whatever the case, is now suggested that the most likely vector for the invasion of the lionfish was the aquarium trade (Hare & Whitfield 2003). The first sighting of a lionfish in The Bahamas occurred in 2004 (Schofield 2009), since then lionfish are thought to have established around every island in the wider Caribbean and along much of the Central and South American coasts (Schofield 2009; Côté et al. 2013). In 2014, lionfish were recorded off the Atlantic coast of Brazil, suggesting that the invasion is continuing southward (Fig. 1.1; Ferreira et al. 2015).

Lionfish are opportunistic predators feeding primarily on smaller fishes, therefore, the loss of native species through direct predation and/or ecological overlap through competition with native fish species has been identified on numerous occasions (Fishelson 1997; Albins & Hixon 2008; Arias-Gonzalez et al. 2011; Albins & Hixon 2013; Albins 2013). Combined with the fact that lionfish experience little fishing mortality, unlike many overfished native species, and experience a generally lower natural mortality due to their venomous spines, lionfish have been shown to have a competitive advantage in their invaded range over native species (Whitfield et al. 2007; Albins 2013). These benefits, coupled with ocean current dispersal and high fecundity, may explain the rapid increase in lionfish abundance since first accounts. It has been suggested that if the spread of lionfish is not managed, their considerable predation pressure, combined with pre-existing stressors, such as overfishing, marine debris pollution and climate change, could cause substantial irreversible changes to coral-reef communities (Albins & Hixon 2013).

The importance of mangrove habitats

Mangrove and back reef ecosystems are vital in the stabilisation of sediments, thereby preventing smothering and scouring of coral reefs (Dahlgren & Marr 2004). They also act as critical buffers between coral reefs and land-based sources of pollutants (Wells & Ravilious 2006). Furthermore, mangroves are critical as nursery and developmental grounds for fish and invertebrates, and provide a net export of economically important species (Faunce & Serafy 2006). For example, Spiny lobster (*Panulirus argus*) and Stone crab (*Menippe mercenaria*) larvae settle from free-floating plankton stages into mangroves and back reef nursery areas, and reside there until they reach adult sizes (Dahlgren & Marr 2004). The critically threatened Queen conch (*Strombus gigas*) relies heavily on mangrove habitats for food and shelter (Dahlgren & Marr 2004). Additionally, recreationally important Bonefish (*Albula vulpes*), commercially valuable, yet threatened, Nassau grouper (*Epinephelus striatus*) and many other species live in mangroves and back reef ecosystems as juveniles before moving onto the flats or coral reefs, respectively, as adults (Dahlgren & Marr 2004). Mangrove and back reef ecosystems also act as critical foraging habitats for many marine predators that rely on the abundance of prey these ecosystems provide for growth and survival. For example, juvenile and sub-adult Lemon sharks (*Negaprion brevirostris*) and Great barracuda (*Sphyraena barracuda*) almost exclusively use mangrove and back reef ecosystems as feeding grounds, as these species are particularly vulnerable as juveniles to predation by larger apex predators (e.g. Tiger sharks (*Galeocerdo cuvier*), Great hammerhead sharks (*Sphyrna mokarran*) and Bull sharks (*Carcharhinus leucas*)) in open ocean environments (Wolff et al. 2000; Laegdsgaard & Johnson 2001; Ellis & Bell 2004).

Lionfish in mangroves

Since the initial sighting of lionfish in the Western Atlantic in 1985, research has focussed on understanding many aspects of lionfish biology both in their native and invasive range (Whitfield & Hare 2003). However, little research has focused on the spread of lionfish beyond coral reef ecosystems. Since 2010, lionfish have been documented, and subsequently studied, in mangrove ecosystems, both in their native and invaded ranges (Table 1.1; Barbour et al. 2010; Prakash et al. 2012; Kulbicki et al. 2012; Jud & Layman 2012; Claydon et al. 2012; Pimiento et al. 2015; Jud et al. 2015). As described above, mangroves provide nursery habitats for numerous fish species (Barbier 2006; Faunce & Serafy 2006; Barbier et al. 2011; Sandilyan & Kathiresan 2015), therefore, the presence of lionfish in these ecosystems is of concern, given the ability of lionfish to consume large quantities of juvenile fishes and invertebrates (Albins & Hixon 2008; Morris & Akins 2009). Subsequently, it is important to understand how lionfish are able to survive in mangrove ecosystems where salinity is often considerably lower in comparison to reef habitats.

Thesis research

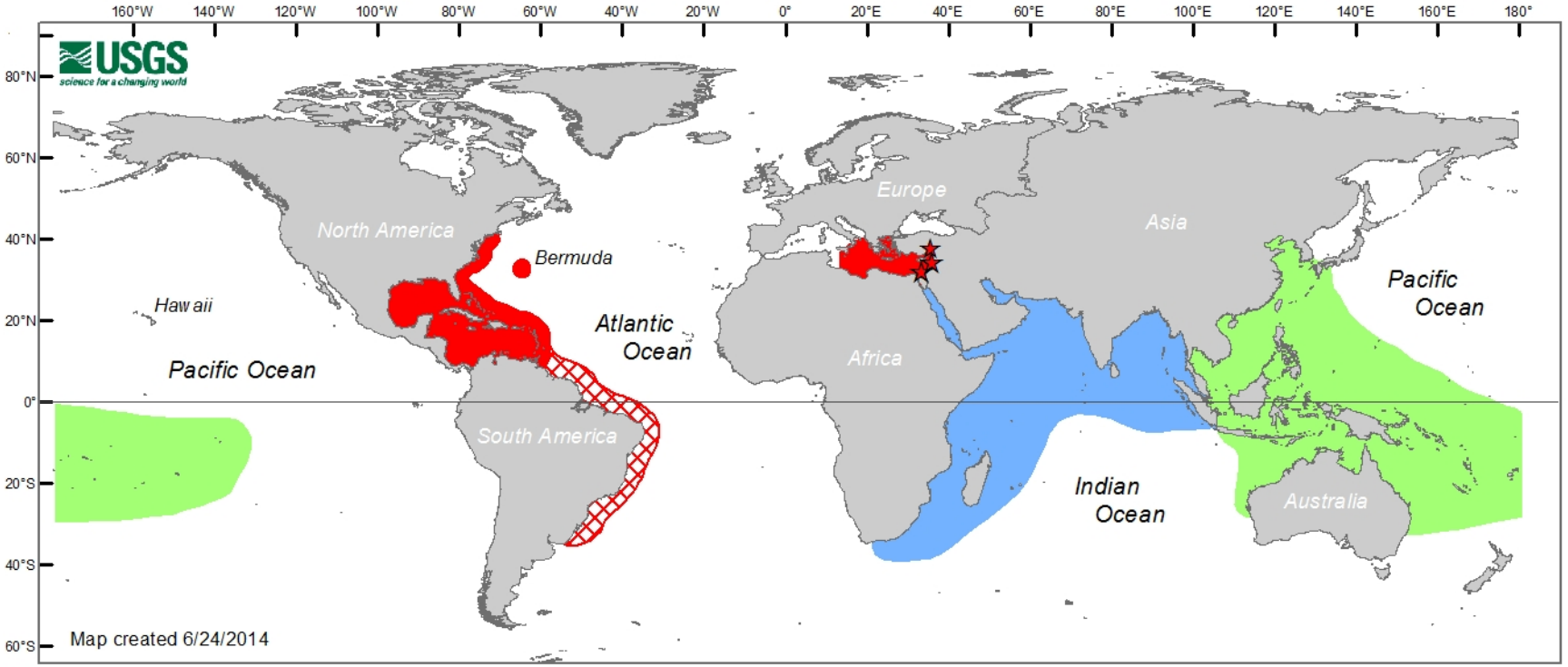
Using a combination of growth, feeding, respirometry and behavioural trials, this thesis explores the potential impacts of the invasive lionfish in low salinity ecosystems. Throughout the thesis, I examine the effects of salinity on the growth, physiology and behaviour of lionfish and, in turn, how their introduction into mangrove ecosystems may affect native species. This research includes the first comprehensive study of lionfish metabolism in low salinity habitats and offers new insights into the bioenergetic effects of low salinity on lionfish. This research also develops some early findings from an experiment

investigating the potential dietary competition between lionfish and Schoolmaster snapper (*Lutjanus apodus*) (Layman & Allgeier 2012). Together, the results provide a better understanding of lionfishes' ability to live in hyposaline waters, the impacts they may have on native species and encourages a re-evaluation of expansion models to include back-reef ecosystems.

Reference	Location	Methodology	Conclusions
Barbour et al. 2010	San Salvador, Bahamas	Stomach analysis	Lionfish colonise and feed in mangrove habitats
Claydon et al. 2012	South Caicos, Turks and Caicos Islands	Compared densities in deep reefs to shallow habitats.	Lionfish are found in mangroves but are more likely in deeper habitats
Jud & Layman 2012	Loxahatchee River Estuary (Florida, USA)	Mark–recapture study	Lionfish showed high fidelity but remained on the outer border of the estuary
Pimiento et al. 2015	San Salvador, Bahamas	Assessment of population sizes and ontogenetic shift from mangroves to reef areas	Populations have increased over the 4 years. No evidence of an ontogenetic shift.
Kulbicki et al. 2012	Multiple locations, Indo-Pacific	Meta-analysis and own population investigation of native range	Lionfish are present in native mangroves

Biggs & Olden 2011	Roatan, Honduras	Combined data collected by citizen scientists with field surveys to provide an examination of habitat occupancy	16% of lionfish identified in seagrass habitats
Jud et al. 2011	Loxahatchee River estuary (Florida, USA)	Description of lionfish capture locations, size ranges, and basic diet information.	Multiple size classes of lionfish (n=211) found in a low salinity habitat
Prakash et al. 2012	Vellar Estuary, Parangipettai	Temporal variations in the abundance of lionfish in the different estuarine locations	Variety of sizes throughout the estuary

Table 1.1 A comprehensive list of publications identifying lionfish in mangroves and other low salinity habitats including: location, methodology and main conclusions



Map of native range of *Pterois volitans* (green) and *P. miles* (blue) adapted from Schultz (1986) and Randall (2005). Stars in Mediterranean Sea denote Lessepsian migration of *P. miles* via the Suez Canal (Golani and Sonin 1992; Bariche et al. 2013; Turan et al. 2014). Non-native range of *P. volitans* and *P. miles* in the Americas is shown in red (from Schofield et al. 2012). Predicted future distribution of lionfish along coastal South America is shown in red hatching (Morris and Whitfield 2009). Lionfish are continuing to expand westward in the Mediterranean Sea (red shading). See Azzurro et al. (2017) for a review.

Figure 1. 1 Map of the native and invasive ranges of *Pterois* spp. Sourced from

https://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=963

Chapter 2: Tolerances of a marine invader: bioenergetic responses of lionfish (*Pterois* spp.) to an environment of lowered salinity.

Introduction

The rapid establishment and successful invasion of the piscivorous lionfish (*Pterois* spp. (Wilcox et al. 2017)*;* hereafter referred to simply as lionfish) in the Western Atlantic and wider Caribbean (Whitfield & Hare 2003; Bax et al. 2003; Snyder & Burgess 2007) is likely one of the most documented and successful marine invasions to date (Côté & Smith 2018). Thought to be the result of intentional (human mediated) releases, the first confirmed sightings of lionfish in the Western Atlantic occurred in Florida, USA, in 1985 (Whitfield & Hare 2003; Morris & Akins 2009). Facilitated likely by a reversal in ocean circulation patterns between Florida and The Bahamas, lionfish reached the Bahamian archipelago in 2004 (Schofield 2009) and have since become established in the waters of every island nation in the wider Caribbean and much of the Central and South American coasts (Schofield 2009; Côté et al. 2013), with individuals even recorded off the Atlantic coast of Brazil (Ferreira et al. 2015).

Lionfish in their invaded range can be found at densities and body sizes that far surpass that of their native range counterparts in the Indian Ocean and the Red Sea (Côté et al. 2013), likely due to a lack of predators and higher survival rates from egg to adult. Paired with voracious feeding habits (Côté et al. 2013), and the ability to exploit the naivety of native reef fish to recognise lionfish as predators (McCormick & Allan 2016), lionfish have been shown to significantly reduce the recruitment and biomass of native reef fish and invertebrates (Albins & Hixon 2008; Green et al. 2012). In a controlled field experiment, Albins and

Hixon (2008) found that lionfish at high densities were responsible, on average, for a 79% reduction in the recruitment of native fish and invertebrate species on patch reefs in the Bahamas, in just five weeks. A longitudinal study over a larger spatial scale was also conducted (Albins 2015). It was found that, through quarterly surveys of patch reefs in the Bahamas, lionfish caused a 46% reduction in total native reef fish densities (Albins 2015). More recent work over a four-year period, before and after lionfish appeared on the Belize Barrier Reef, found no evidence that lionfish affected the density, richness, or composition of prey fish (Hackerott et al. 2017). However, this research has since been disputed as unconvincing and potentially misleading (Ingeman et al 2017).

Recently it has become clear that lionfish are able to colonise a wide variety of habitats including depths in excess of three hundred metres (Albins & Hixon 2013), sea grass beds at depths of less than one metre (Albins & Hixon 2013), and even brackish waters of mangrove and back reef ecosystems (Barbour et al. 2010; Jud & Layman 2011; Jud et al. 2015). Mangrove and back reef ecosystems are vital in the stabilisation of sediments and prevention of smothering and scouring of coral reefs (Dahlgren & Marr 2004). They also act as critical buffers between coral reefs and land-based sources of pollutants (Wells & Ravilious 2006). Furthermore, mangroves are critical as nursery and developmental grounds for fish and invertebrates, and provide a net export of economically important species (Faunce & Serafy 2006). For example, Spiny lobster (*Panulirus argus)* and Stone crab (*Menippe mercenaria*) larvae settle from free-floating plankton stages into mangroves and back reef nursery areas, and reside there until they reach adult sizes (Dahlgren & Marr 2004). The critically threatened and highly cultural and commercial Queen conch (*Strombus gigas*) relies heavily on mangrove habitats for food and shelter (Dahlgren & Marr 2004).

Additionally, recreationally important Bonefish (*Albula vulpes*), commercially valuable, yet threatened, Nassau grouper (*Epinephelus striatus*) and many other species live in mangroves and back reef ecosystems as juveniles before moving onto the flats or coral reefs, respectively, as adults (Dahlgren & Marr 2004). Therefore any change in the trophic balance of these habitats could cause significant declines in many culturally, economically, recreationally and ecologically important species.

Lionfish have been found to tolerate a wider range of salinities than originally thought (Jud et al. 2015), indicating that they may have a greater capacity for range expansion than previously anticipated. Jud et al. (2015) were among the first to report not only the presence of lionfish in hyposaline environments as far as 6.6 km from the ocean but also the ability of lionfish to tolerate low salinity conditions, with 15 out of 16 individuals surviving for more than 28 days in a salinity treatment of only 7 ppt (relative to approximately 37 ppt in a normal marine reef environment in the Caribbean; Jud et al. 2015).

Salinity varies spatially and temporally among and between aquatic habitats such as rivers, estuaries, oceans and mangroves. Some fish species transition between habitats of differing salinities daily (Grey Mullet *Chelon labrosus*; Snook *Centropomous undecimalis*) (Cardona 2006)), during certain developmental stages (various salmonids *Oncorhynchus* spp.) (Quiñones & Mulligan 2005)) or have evolved to carry out their complete lifecycle in habitats of contrasting salinities (Three Spined Stickleback *Gasterosteus aculeatus*; Killifish *Fundulus heteroclitus*) (Morgan & Iwama 1991)). Whatever the case, research has shown that many species of fish have evolved a means of osmoregulation that involves interplay between being hyper-osmotic in fresh water, to being hypo-osmotic in marine environments, with the degree

determining a species tolerance (euryhaline vs. stenohaline) (Morgan & Iwama 1991).

Although life history plays a significant role in the salinity tolerance of a species, the physiological capacity of a species may be important too (Davidson et al. 2011). The movement of osmotic solutes across a gradient is generally regarded as being energetically expensive, and may constitute upwards of 20 to 50% of the total energy budget in fish (Boeuf & Payan 2001). Therefore, energy compensation should occur in secondarily important physiological traits related to growth, reproduction, food consumption, locomotory capacity and digestion (Boeuf & Payan 2001). It might be expected that fish in brackish waters will have a lower energetic cost associated with osmoregulation compared with those in salt or fresh water (Boeuf & Payan 2001). However, the majority of fish species are specialised to either hyper or hypo-osmoregulate with a limited ability to do both, suggesting limited dispersal across both marine and freshwater ecosystems (Evans 1984). In species where this does occur it has been suggested that a period of 3 - 12 days are needed to acclimate from salt to fresh water with the process being costly and sometimes fatal (Ferraris et al. 1988; Nonnotte & Truchot, 1990; Jensen et al. 2002; Sampaio & Bianchini 2002). Therefore, success of a species to utilize variable salinity habitats is dependent on the speed of acclimation and ability to maintain biological function thereafter. It has also been suggested that considerable plasticity within a species to both hyper and hypo-osmoregulate also exists (Christensen et al. 2019).

While relatively few species have a broad capacity to maintain constant body fluid and ion composition within both dilute and concentrated environments, due to the expensive process of ion loss and diffusion (Serrano 2008), this has not specifically been tested with lionfish. However, due to their native range,

typical reef association and the general inability of most fish species to osmoconform (organisms that maintain an internal salinity similar to their ambient conditions), it is assumed that lionfish are osmoregulators, (actively pump ions when in more saline water to combat water loss to the environment; Marshall & Grosell 2005).

Physiological traits that are commonly measured in energetics research and likely important in determining the salinity tolerance of a species include: Standard metabolic rate (SMR), the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (Chabot et al. 2016), maximal metabolic rate (MMR), the rate of oxygen consumption during the maximum sustainable rate of exercise, and aerobic scope (AS), the difference between an animal's SMR and its MMR, so that AS defines the capacity of an animal to increase its rate of aerobic metabolism (Norin et al. 2014). Together, these metabolic traits make up what is termed the metabolic phenotype of an individual, and are important factors associated with the anabolism and catabolism of tissues, organism homeostasis, digestion (specific dynamic action (SDA) the peak in oxygen consumption following a meal), lifestyle and behaviour (*e.g*. benthic *vs*. pelagic) (Brown et al. 2005; Killen et al. 2010).

The effect of salinity on the growth of juvenile and adult fish has been studied in 'true' marine species (i.e. Atlantic cod; *Gadus morhua*) and freshwater species (i.e. carp) (Boeuf & Payan 2001). As growth is continuous in fish, it is predicted to be one of the first processes affected during stressful environmental situations (Boeuf & Payan 2001). A review of literature on fish growth in varying salinities identified that higher growth rate is often observed at intermediary salinity (Boeuf & Payan 2001). In most cases this occurs because of a decrease in metabolic cost as a result of the body being isotonic with the surrounding

environment and no requirement to osmoregulate. Even species which are considered 'true' marine species, such as cod (*Gadus morhua*), or turbot (*Scophthalmus maximus*), have growth rates significantly increased at intermediate salinity conditions of 12 to 19 ppt (Lambert et al. 1994; Gaumet et al. 1995; Dutil et al. 1997; Imsland et al. 2001; Boeuf & Payan 2001). As lionfish are typically considered a true marine species, understanding their growth and physiological capacity in intermediate salinity conditions such as those often experienced in mangrove ecosystems, is pivotal to understanding impacts of lionfish in mangroves.

The present study used a series of laboratory experiments to investigate the effect of lowered salinity on lionfish growth, maximum food consumption, metabolic rate and digestion. As previous research has indicated that lionfish are able to survive in low salinities for extended periods, our objectives were four-fold; 1) to calculate the daily growth rates of lionfish when acclimated to different salinity treatments during a period of both low and high food availability; 2) determine how metabolic traits varied under different salinity treatments; 3) to determine whether lionfish are able to merely physiologically tolerate versus live comfortably in low salinity environments; and 4) to gain an insight into the potential physiological impact on lionfish during their expansion into mangrove ecosystems.

Methods

Animal capture and transport

Lionfish were collected by divers on SCUBA using clear plastic hand nets from patch reefs (~ 3 m depth) located adjacent to the Cape Eleuthera Institute (CEI), The Bahamas (24°50′ N; 76°20′ W). Patch reefs were located using a

handheld GPS (Garmin eTrek, Kansas, USA) and known coordinates from a previous lionfish study conducted at CEI (Smith et al. 2017). Once captured, lionfish were transported by boat in a large cooler filled with seawater to aquarium holding facilities at CEI. An air stone was placed in the cooler and the seawater changed periodically to ensure adequate air saturation during transport. On 20 February 2017, all fish were fasted for 48 hours, anaesthetised using clove oil (20 mg / L) (NRC 2010), measured (total length and standard length to the nearest mm), weighed (to the nearest 0.1g) and externally tagged (Floy tag Inc, Washington, USA) in the dorsal musculature so that each fish could be individually identified throughout the duration of the experiment.

Lionfish husbandry and feeding

Lionfish (n = 66) were equally divided across six 750 L aquaria (160 cm diameter X 60 cm depth) and supplied with flow-through seawater at ambient temperature (mean 25.0 ± 2.7 °C) and salinity (mean 37.0 ± 2.3 ppt). All six aquaria contained a similar size distribution of lionfish (mean mass = 110.24 ± 6.92 g; mean length = 198.55 ± 1.96 mm). Aquaria temperature, salinity and water flow were checked daily and cleaned using a vacuum siphon and scrub brush to remove any debris accumulating on the bottom. All lionfish were left for one week after capture and tagging to allow for settling and recovery. All lionfish were in good external condition and on a natural day / night cycle prior to experiments.

Lionfish were fed live Atlantic silversides (*Menidia menidia*) at either (i) low food availability (1.5% of individual lionfish's body weight every four days from 17 March 2017 to 10 April 2017 (Jud et al. 2015)) or (ii) high food availability (fed *ad libitum* daily for two weeks from 7 May to 24 May 2017). Each lionfish was used as a repeated measure for both feeding trials due to the length of time required for acclimatisation to salinity treatments and captive feeding. During feeding,

individual lionfish were removed from their holding aquarium using plastic hand nets, identified using the external tag and placed in a feeding arena (plastic container 63 X 40 X 35 cm) floating within the main aquarium. Individuals from each of the six holding aquaria were done individually. This was done to ensure all lionfish were fed and consumed their allocated 1.5% body weight without competition and disturbance from other lionfish during the low food availability experiment. Although netting disturbance was not specifically tested as an effect of lionfish feeding, any effect would have been spread across the treatments given the standardised feeding regime. Also, given that all fish ate large quantities of food throughout the duration of the experiment, it is assumed that any disturbances would be minimal, as feeding often ceases in stressed fish (Wendelaar Bonga 1997; Schreck et al. 1997; Santos et al. 2010), which was certainly not the case in our study. Once a lionfish consumed all allocated food, it was removed and placed in a temporary post-feeding arena (plastic container 63 X 40 X 35 cm) also floating within the main aquarium. After feeding, all lionfish were released back into their main aquarium. During the low food availability feeding sessions, the time taken for each lionfish to consume all prey offered was recorded (min.), and the amount of prey consumed was recorded (g). We iteratively determined that lionfish would normally consume all prey offered within a five-minute period. Therefore, if a lionfish did not consume their allocated food during this five-minute period, a feeding duration of five minutes was recorded and the lionfish placed in the post-feeding arena. Maximum food consumption was determined in a similar process, except there was no time limit and prey were introduced at a greater quantity (5% of the fish's body weight at a time). This continued until signs of satiation were observed (food was regurgitated, lionfish seemed uninterested with additional input of prey by moving away, and no

predatory behavioural displays e.g. fin display (Lönnstedt et al. 2014) for a continuous five-minute period. After satiation, any remaining prey were removed from the tank, blotted dry, weighed, and subtracted from the total weight of prey given so that maximum food consumption could be calculated.

Salinity treatments

Lionfish were acclimated to three salinity treatments (low / 10 ppt, mid / 20 ppt and control / 37 ppt), similar conditions to those lionfish might be exposed to moving from a coral reef (37 ppt) to a mangrove habitat (20 ppt to 10 ppt) (Primavera 1998). Salinity of the treatment aquaria was lowered over five days (by 5 ppt every 24 hours between 27 February and 3 March 2017) (Jud et al. 2015) by the addition of fresh water. The flow rates of fresh water and salt water were iteratively determined to give experimental salinities of 20 ppt and 10 ppt, giving two replicates of both a low and mid salinity treatment, and two replicates of a control treatment (37 ppt). Water changes were conducted daily at 09:00, with an input of flow-through fresh and salt water for 1.5 hours. Adequate mixing was achieved using an air stone and by ensuring the fresh water intake was located adjacent to the bottom of the aquaria. The salinity and temperature of the in-flowing sea water and fresh water were measured prior to water changes to ensure minimal disturbance. Fish were left for one month to acclimate to altered salinities. Salinity was checked daily using a refractometer calibrated using distilled water.

Measuring metabolic rates

After approximately one month in hyposaline conditions (experiments started between 14 April 2017 and 3 May 2017) all fish were subjected to respirometry measurements (Fig. 2.1). Eight lionfish were placed into separate plastic respirometry chambers (23 X 19 X 22 cm; chamber volume = 9614 ml).

Chambers were submersed in a seawater bath maintained at the salinity lionfish were acclimated to and at a constant temperature (24.0 ± 1.0 °C) across all experiments. An air-stone in the seawater bath of the respirometer apparatus ensured in-flowing water was fully air saturated. Oxygen consumption was measured using intermittent flow-through respirometry (Steffensen 1989) controlled using an automated respirometry system which consists of software, solenoid and oxygen optodes (AutoResp, Loligo, Viborg, Denmark; Fig. 2.1). Once a fish was placed in the respirometer chamber, the flush pump (Eheim 300 universal, Deizisau, Germany), which delivered oxygenated water to the respirometer, was immediately stopped using software controlled solenoid valves. The oxygen in the respirometry chamber then decreased due to the lionfish respiring (oxygen saturation in the chambers was monitored and never dropped below 90% O_2). Thereafter, the oxygen concentration in the chamber was measured using one of eight fibre-optic oxygen probes (Witrox 1, Loligo Systems) every second for 20 minutes. Oxygen probes were calibrated using a sodium sulphite solution and air-saturated water (0% and 100% settings, respectively). Flush pumps were programmed to switch on for six minutes to restore oxygen saturation levels before the start of the next measurement cycle. Data were processed using AutoResp software (Version 1.4, Loligo, Viborg, Denmark). Baseline oxygen concentration was corrected for ambient temperature, salinity and barometric pressure, which were inputted into the software prior to the respirometry trial.

The rate of oxygen consumption was determined using the following equation (Ege & Krogh 1914):

$$MO_2 = \frac{V_W . \Delta C_W O_2}{\Delta t}$$

where V_w is the volume of water in the respirometer, ΔC_wO_2 is the change in oxygen tension of the water, and Δt is the time period associated with the drop in oxygen tension in the respirometer (Steffensen 1989). The coefficient of determination (r^2) for all slope measurements (oxygen concentration vs. time) were greater than 0.95. The effect of background levels of oxygen consumption (e.g. by bacteria in the water) for each specific fish and chamber was corrected by measuring the oxygen concentration in the absence of fish at the beginning and end of each measurement trial. Following respirometry measurements, all water was changed and the respirometry apparatus cleaned using a bleach solution to ensure bacterial respiration was kept to a minimum. Chambers were thoroughly rinsed to ensure the full removal of the bleach solution. Food was withheld from the eight fish intended for respirometry experiments for at least 48 hours prior to measurements, to ensure sufficient time was given for fish to evacuate their gut; 20h post-feeding has been shown to be adequate for the specific dynamic action (SDA) response to subside (McCarthy 2000; Cutts et al. 2002; Van Leeuwen et al. 2012).

Measuring maximal metabolic rate (MMR)

To determine MMR, individual fish were sequentially subjected to an exhaustive chase protocol (Reidy et al. 1995; Killen et al. 2010; Norin et al. 2014), where a single fish was introduced into a rectangular arena (60 L) and hand-chased with a small net to exhaustion. Exhaustion behaviour included the fish having ceased swimming when touched and increased gill activity. This generally occurred after four minutes. Once exhausted, individuals were immediately placed into one of eight separate plastic respirometry chambers, as described above. Maximal metabolic rate was determined as the highest oxygen

consumption rate of all measurements represented in the data. In all cases, this value corresponded to the first measurement immediately following the exhaustive chase protocol.

Standard metabolic rate (SMR) and determination of aerobic scope (AS)

Once MMR measures had been completed, the fish remained in the same respirometry chambers for 24 hours to allow for measurements of SMR (minimal metabolic rate in ectotherms). A plastic divider was placed between each respirometer to prevent visual contact between individual fish during measurements. Furthermore, all measurements were conducted in the dark to further minimise fish disturbance (Cutts et al. 2002). Human disturbance through noise and entry to the respirometry laboratory was also kept to a minimum. Whole-animal SMR (mg O_2 h^{-1}) was estimated as the average of the lowest 10th percentile of SMR measures taken throughout the measurement period, excluding the first five hours when oxygen consumption of fish was often elevated due to activity over this time (Steffensen 1989; Chabot et al. 2016), and expressed in mass specific values. Aerobic scope was calculated post experiment as the difference between MMR and SMR.

Specific dynamic action (SDA)

To measure SDA, lionfish were fed 1.5% of their body weight of live silversides, after the completion of MMR and SMR measures. Live silverside prey were introduced through a porthole in the respirometer chamber, which was plugged with a rubber stopper during oxygen consumption measurements. During feeding, the flush pump and recirculation pump were stopped to prevent silverside prey from entering the respirometry tubing. All meals were generally consumed within two minutes of being introduced into the respirometer. Lionfish that did not consume all the food offered whilst in the respirometer were excluded

from the analyses (n = 4). On completion of the first postprandial oxygen consumption reading, all lionfish had consumed the entire meal. Oxygen consumption was recorded until it returned to pre-feeding levels (which generally took approximately 48 h (Penney et al. 2016; McGaw & Van Leeuwen 2017)). Specific dynamic action was enumerated using four metrics: 1) SDA_{Max}, peak oxygen consumption recorded after feeding, 2) SDA_{Scope}, difference between the peak in oxygen consumption following feeding and baseline oxygen consumption prior to feeding, 3) SDA_{Total}, the total increase in oxygen consumption above baseline, standardised to kJ using the conversion factor of 1 mg O_2 = 0.014 kJ (Secor 2009; McGaw & Van Leeuwen 2017) and finally 4) $SDA_{Duration}$, total time elapsed between the first increase in oxygen consumption after feeding and return of oxygen consumption to pre-feeding levels. These metrics were calculated post experiment using KaleidoGraph (McGaw & Van Leeuwen 2017).

Calculations and statistical analyses

Instantaneous growth rates of fish (% body length / day) were calculated following (Ricker 1975):

$$\frac{[\log(\text{final length}) - \log(\text{initial length})]}{\text{Time (days)}} \times 100$$

Average time to consume all food (prey capture efficiency) was calculated during the low food availability portion of the experiment by averaging half of the times taken for each fish to consume all allocated food during all feeding bouts. We tested for the effects of salinity treatment and individual fish mass on growth, prey capture efficiency, maximum food consumption, MMR, SMR, AS, SDA_{Max}, SDA_{Scope}, SDA_{Total} and $SDA_{Duration}$. Visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity and a Shapiro-Wilks test identified normality. Models were compared using AIC criterion before and after

removal of interaction terms and independent variables that were not significant at $p < 0.05$. Values for growth, prey capture efficiency, maximum food consumption, MMR, SMR, AS, SDA_{Max}, SDA_{Scope}, SDA_{Total} and $SDA_{Duration}$ were used with mass as a covariate because of the strong but somewhat predictable influence of mass on the metrics of interest. All analyses were conducted using RStudio Desktop 1.2.1335 – © 2019, Inc. statistical software (RStudio Team 2019).

Ethics

All work was carried out under the Bahamas Department of Marine Resources permit number MAMR/FIS/17 and gained ethical approval from the University of Exeter reference number, 2017/1760. As lionfish are an invasive species in the Atlantic and wider Caribbean (Whitfield & Hare 2003), subjects could not be released back to the wild after experiments, and were instead euthanised using a lethal solution of water and clove oil, a widely accepted method of fish euthanasia as suggested by The Guide for the Care and Use of Laboratory Animals (NRC 2010).

Results

Growth, average prey consumption time and maximum food consumption

There was no significant difference in growth in body length between fish from the 10 ppt, 20 ppt and 37 ppt treatments during the low food availability portion of the experiment ($F_{5, 60} = 1.04$, $p = 0.40$; Table 2.1; Fig. 2.2A), with fish growing 0.016, 0.027 and 0.016 $cm.day^{-1}$ respectively (mean value). However, when food was increased to high availability, fish from the control treatment (37 ppt) grew significantly faster than the fish from the 10 ppt treatment: 0.325 versus 0.187 $cm.day^{-1}$ respectively ($F_{2, 26} = 15.90$, $p < 0.05$; Table 2.1; Fig. 2.2B). There

was no significant difference in average time to consume all food (prey consumption time) between treatments, 10 ppt, 20 ppt, and control (37 ppt) (89.55, 117.62 versus 129.80 seconds respectively ($F_{2, 60}$ = 2.23, p = 0.12) but there was a significant difference in the total amount of food consumed, with fish from the 10 ppt treatment consuming less than fish from the 20 ppt treatment (5.68g versus 8.65g respectively (mean value); $F_{2, 44}$ = 3.95, p= 0.03; Table 2.1; Fig. 2.3C)). There was no difference found in the 37ppt treatment compared with 10 ppt or 20 ppt treatments.

Standard metabolic rate, maximal metabolic rate and aerobic scope

There was no significant difference in SMR between lionfish from the 10 ppt, 20 ppt and 37 ppt (control) treatments. However, lionfish from the 10 ppt and 20 ppt treatments had a significantly lower MMR ($F_{3, 36}$ = 192.10, p < 0.05; Fig. 2.3A) and AS ($F_{3, 36}$= 130.40, p < 0.01; Fig. 2.3B) than fish from the 37 ppt treatment (Table 2.1). There was no significant difference in MMR and AS between fish from the 10 ppt and 20 ppt treatments.

Specific dynamic action

There was no significant difference in SDA_{Max}, SDA_{Scope} or SDA_{Total} among fish from the 10 ppt, 20 ppt and 37 ppt treatments. However, lionfish from the 10 ppt treatment had a significantly increased SDA duration than lionfish in the 37 ppt (control) treatment (mean of 34.4 versus 25.9 hours respectively; $F_{3, 32}$ = 5.40, p < 0.01; Table 2.1; Fig. 2.3D) but not among any other treatments.

Mortalities

Eleven lionfish from the 20 ppt treatment succumbed to an infection, assumed to be parasitic, between 12 April and 18 April 2017. A further eleven lionfish from the 20 ppt treatment and fifteen lionfish from the control treatment succumbed to the same infection during the high food growth portion of the

experiment (7 May to 24 May 2017), approximately three weeks after the low food growth and respirometry measures were concluded. While it is unclear what triggered the infection within these treatments, no mortalities occurred in the 10 ppt treatment.

Discussion

Consistent with Jud et al. (2015), we found that lionfish can survive in low salinity conditions (10 ppt) for at least 2 months. Coping with osmotic gradients (e.g. in hypo- and hypersaline environments) is generally regarded as being energetically expensive (Webb 1975; Stevens & Dizon 1982; Febry & Lutz 1987), drawing energy away from other important processes, such as growth or metabolic rate, particularly when food rations are limited. In the present study, this appeared to be the case when food was abundant, but we found no evidence that hyposalinity negatively affected growth rate or SMR in lionfish at low food availability. This is surprising, as changes in SMR in low salinity environments have been demonstrated in other species. For example, Morgan and Iwama (1991) found that SMR increased as salinity increased in Steelhead (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*), and Dalziel et al. (2012) found a significantly lower MMR and AS in sticklebacks (*Gasterosteus aculeatus*) in hyposaline conditions, indicating the potential for some physiological impairment (Morgan & Iwama 1991; Dalziel et al. 2012). In contrast to this and similar to the present study, Grøtan (2012) found no difference in SMR in Threespine sticklebacks among salinity treatments and concluded that these fish may be able to move among varying salinity environments without large short-term metabolic costs, irrespective of their environment of origin (Grøtan et al. 2012). Differences in MMR and AS are

usually discussed in relation to cardiovascular ability and swim performance. However, in the present study, the reduction in MMR and AS may be due to longer digestion, lower maximum food consumption, and decreased growth rate as fish in the 10 ppt treatment ate less food and grew more slowly than control conditions during high food availability. After consuming a meal, SDA can constitute up to a 136% increase in the maximum rate of oxygen consumption of a fish (Alsop & Wood 1997; Secor 2009), and can last anywhere from three to one hundred and eighteen hours depending upon the species and water temperature (Secor 2009). For example, in relatively sedentary fish species such as catfish, the peak in oxygen uptake following feeding can exceed that observed during peak aerobic exercise (Fu et al. 2005). An increase in peak MO_2 (VO_2 in terrestrial animals) with meal size has also been found in Atlantic cod (*Gadus morhua)* (Soofiani & Hawkins 1982) and the Marine toad (*Bufo marimus)* (Secor & Faulkner 2002). In the present study, the magnitude of SDA (SDA_{Max}) did not differ among treatments, but did last a third longer in the lowest hyposaline treatment ($SDA_{Duration}$). Therefore, the results of the present study suggest that lionfish may have developed alternative means of coping with the increase in oxygen consumption brought about by feeding (i.e. extending how long digestion takes, depending on the salinity of their ambient conditions). In low salinity environments, lionfish may trade-off eating less food with longer SDA duration to increase their digestive efficiency and potentially compensate for the added cost of ion regulation at the lowest salinities and shortage of the next available prey. This does not explain why digestion time was similar between the 37 ppt and 20 ppt treatments, especially considering lionfish should have been closer to isotonic state at 20 ppt than 37 ppt. An alternative explanation could be that low salinity

simply slowed gut contractions in the lionfish or reduced gastric activity, which has been shown previously in the Blue crab (McGaw 2006).

The growth of a typical marine species, Atlantic cod (*Gadus rnsrhua*), has also been investigated at three different salinities (7, 14, and 28 ppt) and two feeding levels (Lambert et al. 1994). Results indicated a significant effect of salinity and food ration on growth rate. Growth rates were highest for cod in intermediate salinity conditions (14 ppt) (Lambert et al. 1994). However, growth rates at lower salinities were greater than in high salinity during high food (Lambert et al. 1994). This is different to what was found in the present study, suggesting a difference in resource allocation and conversion efficiency and that lionfish may manage physiological trade-offs differently than other marine fish species.

It is assumed that most fish are specialised to either hyper or hypo-osmoregulate and will rarely do both due to the expense of moving through these environments (Serano 2008). However, certain species do spend their life cycle moving between salinity regimes (Christensen et al. 2019). The physiological responses through blood analysis in marine teleost's after sudden transfer to different salinity treatments has been investigated (Serano 2008). A further development of the present study could look at both the blood of the lionfish within varying salinities but also how the lionfish respond during acute changes rather than after acclimation. Even conducting respirometry on a lionfish who has experienced an acute change in salinity would be an advantageous development and may help determine whether eggs and larvae are passively drifting and developing in mangrove habitats or whether juveniles are actively migrating into mangrove habitats.

The results of the present study have ecological implications for modelling lionfish range expansion, suggesting that lionfish have considerable potential to further invade mangrove and back reef ecosystems. Given the ability of lionfish to survive in low salinity (Jud et al. 2015), we suggest that range expansion models should begin to incorporate hyposaline ecosystems. By not incorporating salinity tolerance, the results of these models may underestimate future range expansions, although the interaction between temperature, salinity and other abiotic factors remain untested. Mangrove and back reef ecosystems are known to be important nursery habitats for juvenile fish species (Faunce & Serafy 2006; Barbier 2006; Mateo et al. 2010; Barbier et al. 2011; Sandilyan & Kathiresan 2015), but results suggest that lionfish may consume less prey and have slower digestive processes, and decreased growth, despite being able to survive at the lowest of salinities tested in the present study. This indicates that the impacts may not be as negative as those experienced previously for some reef ecosystems, where lionfish have been shown to feed voraciously on at least 167 vertebrate and invertebrate prey species across multiple trophic guilds (Morris & Akins 2009; Peake et al. 2018).

In the present experiment, there were several mortalities within the 20 ppt and 37 ppt treatments, however, no mortalities occurred within the 10 ppt treatment. Parasites of invasive marine invertebrates have been relatively well studied (Byers 200; Torchin et al. 2001, 2005) and until recent years very little was known about parasites in marine vertebrates including those that infect lionfish (Tuttle et al. 2016). Tuttle et al. (2016) identified that lionfish were 18 times less likely to host a parasite in The Bahamas compared with sympatric, native fishes. However, the results of our study suggest that lionfish may succumb to parasitic infections outside their native range. Because no mortalities were

observed at 10 ppt, parasites may not have been able to survive at the lowest of salinity treatments, suggesting a further benefit for lionfish inhabiting mangroves. It would be beneficial for the relationship between parasitic infection and salinity tolerance in lionfish to be further investigated.

In a natural mangrove ecosystem, lionfish would experience an influx of high salinity water (37 ppt) during the flood tide, which would be replaced with low salinity water (10 ppt) during the ebb tide, as the flow of water changes and moves seaward. Therefore, lionfish in the upper reaches of mangrove habitats may experience salinity fluctuations of ~ 27 ppt every six hours (Diele & Simith 2006). Notably, some mangroves do not have a consistent freshwater input and thus have reduced salinity at low tide. Therefore, the challenges of living in low salinities may not be applicable to all mangroves globally as elevated salinities may prevent invasions, although this remains to be tested. Although the present study has shown that lionfish can survive in a stable hyposaline environment, a future area of research would be to investigate how lionfish cope with rapid changes in salinity and under hypersaline conditions.

Another interesting development in the study of lionfish invasion in mangrove ecosystems could be to build upon the work investigating the movements of lionfish (Jud & Layman 2012) to determine if lionfish are passively entering mangroves through the dispersal of eggs and larvae or actively moving into mangroves. Therefore, it would be interesting to investigate not just site fidelity, but how and when lionfish are moving and how salinity changes during this movement (e.g. using bio-logging; (Hussey et al. 2015)). One possibility is that lionfish may not simply reside in mangroves; instead, they may be travelling in on flowing tides to feed intermittently during high tide cycles, and then moving back onto the reef habitat, although this remains unknown and unlikely given the

energetic cost associated with such a feeding strategy. Nevertheless, this movement would allow lionfish to exploit the high juvenile fish populations found in mangroves before returning to optimum salinity, which may allow for maximum digestive efficiency. More in-depth surveys of mangrove ecosystems for the presence of lionfish would be useful in providing further insight, as mangroves remain a relatively under-investigated habitat, and the presence of lionfish in these ecosystems is likely being under-reported (Barbour et al. 2010; Claydon et al. 2012; Pimiento et al. 2015).

Figures and Tables

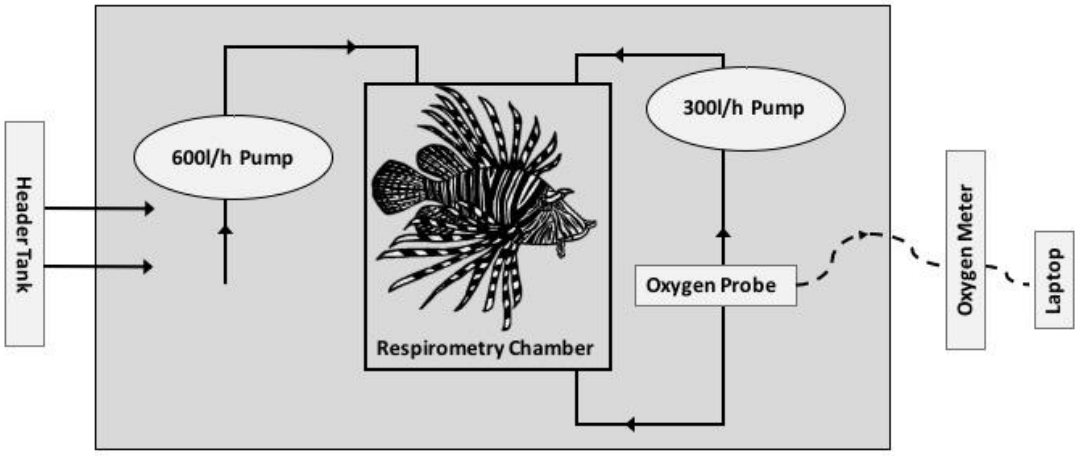


Figure 2.1 A schematic of the intermittent-flow respirometry set-up used to measure oxygen consumption, and determine the metabolic phenotype and digestive efficiency of individual lionfish. Solid lines indicate flow of water; dotted lines indicate flow of information.

Lionfish image sourced from http://minarium.club/full/

	Low food			High food	
Metric	Control (37 ppt)	20 ppt	10 ppt	Control (37 ppt)	10 ppt
Sample size of lionfish	22	22	22	10	19
Growth (% body length / day)	0.016 ± 0.094	0.028 ± 0.011	0.016 ± 0.005	0.325 ± 0.026	0.187 ± 0.019 (b)
Prey capture efficiency (sec)	129.80 ± 19.27	117.62 ± 15.87	89.55 ± 17.36		
Sample size of lionfish	18	10	22		
Maximum food consumption (g)	5.91 ± 0.69	8.05 ± 0.91 (c)	5.68 ± 0.44		
Sample size of lionfish	13	11	16		
MMR (mg O_2 / g / h)	0.213 ± 0.008	0.181 ± 0.009 (a)	0.166 ± 0.008 (b)		
SMR (mg O_2 / g / h)	0.074 ± 0.004	0.068 ± 0.004	0.077 ± 0.003		

AS (mg O_2 / g / h)	0.139 ± 0.007	0.113 ± 0.006 (a)	0.089 ± 0.008 (b)
Sample size of lionfish	11	10	15
SDA_{Max} (mg O_2 / g / h)	0.133 ± 0.008	0.112 ± 0.01	0.136 ± 0.01
SDA_{Scope} (mg O_2 / g / h)	0.055 ± 0.006	0.045 ± 0.006	0.058 ± 0.008
SDA_{Total} (kJ)	0.880 ± 0.187	0.820 ± 0.28	1.180 ± 0.361
$SDA_{Duration}$ (hrs)	25.890 ± 0.676	29.850 ± 1.876	34.350 ± 1.862 (b)

Table 2.1 Experiment values for lionfish acclimated to three salinity treatments (low / 10 ppt and 20 ppt and control / 37 ppt). Values represent the mean ± SE; (a) indicates metrics that were statistically significant between 20 ppt and control, (b) indicates metrics that were statistically significant between 10 ppt and 37ppt, and (c) indicates metrics that were statistically significant between 20 ppt and 10 ppt, all significance is at 5%.

NB: Due to unexpected mortalities during the high food portion of the growth experiment only data from the 10 ppt and 37 ppt treatments were compared.

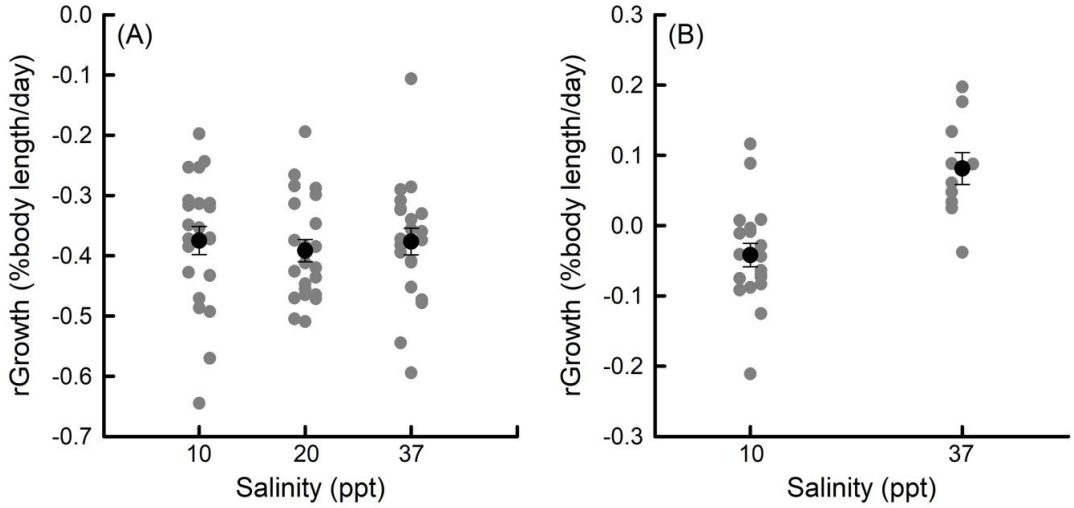


Figure 2.2 The relationship between salinity (low / 10 ppt and 20 ppt and control / 37 ppt) and mean growth rate residual corrected for body mass (rGrowth; % body length / day) for; **(A)** lionfish fed a low food ration for 24 days and **(B)** lionfish fed on a high food ration for 14 days. Dark points show the mean growth for each treatment, error bars represent ± SE and faded points show all data collected. Residuals were calculated from the regression equation of growth (% length / day) against body mass (g) for all fish.

NB: Due to unexpected mortalities in the 20 ppt treatment during the high food ration experiment only fish from the 10 ppt and 37 ppt treatments are compared **(B).**

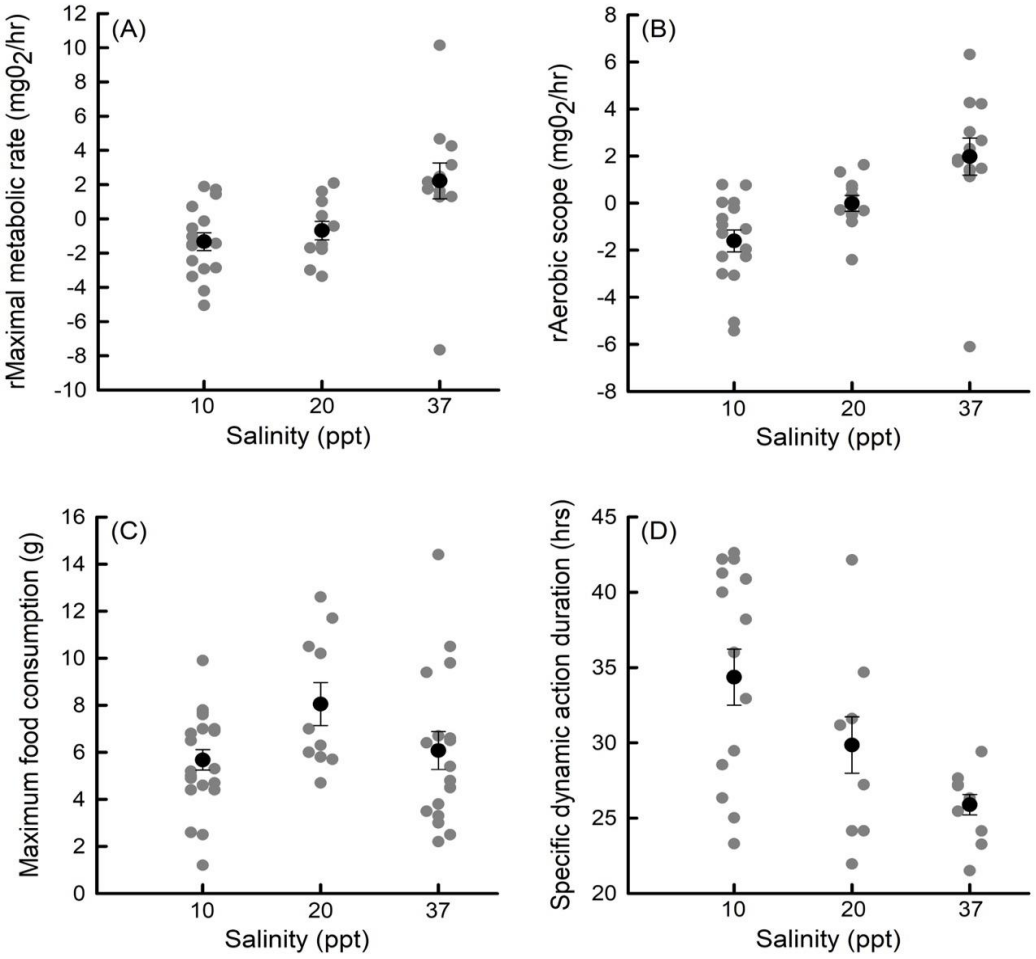


Figure 2.3 The relationship between salinity (low / 10 ppt and 20 ppt and control / 37 ppt) and metabolic rate for; **(A)** maximum metabolic rate (residual corrected for body mass (MMR; mg O_2 / h)) for lionfish (n = 40), **(B)** aerobic scope (residual corrected for body mass (rAS; mg O_2 / h)) for lionfish (n = 40), **(C)** maximum food consumption (g) for lionfish (n = 50) and **(D)** the duration of the specific dynamic action response ($SDA_{Duration}$; in hrs) for lionfish (n = 36).

Residuals for MMR and AS were calculated from the regression equation of the whole values (mgO_2 / hr) against body mass (g) for all fish. Dark points represent the mean value for each treatment, error bars represent ± SE and faded points represent all data collected.

Chapter 3: The lion, the fish, and the mangrove: interactions between Schoolmaster snapper and invasive lionfish in a simulated mangrove ecosystem

Introduction

Invasive species are a widely-recognized threat to regional and global biodiversity and are quickly becoming one of the most severe environmental crises as impacts following successful colonisation can be catastrophic (Wilcove & Chen 1998; Clavero & García-Berthou 2005). Invasive species can have lasting detrimental effects on the ecosystems they invade directly by removing native plant and animal species through grazing or predation, out-competing native species or physically altering the environment (Bax et al. 2003; Galil 2007; Molnar et al. 2008). Indirectly, invasive species can introduce foreign diseases, with devastating results to local taxa (Crowl et al. 2008) and cause significant detrimental impacts on human health (Juliano & Lounibos 2005; Pejchar & Mooney 2009).

The piscivorous lionfish (*Pterois* spp. (Wilcox 2017)*;* hereafter referred to simply as lionfish) are an invasive marine fish in the Western Atlantic and wider Caribbean (Côté et al. 2013). The majority of research has shown that lionfish significantly reduce the recruitment and biomass of native reef fishes (Albins & Hixon 2008; Green et al. 2012; Albins 2015). Thought to be the result of intentional (human mediated) releases, the first confirmed sightings of lionfish in the Western Atlantic, occurred in Florida, USA, in 1985 (Whitfield & Hare 2003; Morris & Akins 2009). Thereafter, likely facilitated by a reversal in ocean circulation patterns between Florida and The Bahamas, lionfish reached the

Bahamian archipelago in 2004 (Schofield 2009) and have since become established in the waters of every island nation in the wider Caribbean and much of the Central and South American coasts (Schofield 2009; Côté et al. 2013). Recently it has become clear that lionfish are able to colonise a wide variety of habitats including depths in excess of three hundred metres (Albins & Hixon 2013), sea grass beds at depths of less than one metre (Albins & Hixon 2013), and even brackish waters of mangrove and back reef ecosystems (Barbour et al. 2010; Jud et al. 2011; Jud & Layman 2012; Jud et al. 2015).

Mangroves are critical as nursery and staging grounds for fish and invertebrates, and provide a net export of economically important species for recreational and commercial fisheries (Faunce & Serafy 2006; Barbier 2006; Mateo et al. 2010; Barbier et al. 2011; Sandilyan & Kathiresan 2015). In addition, mangrove and back reef ecosystems act as critical foraging habitats for many marine predators which rely on the abundance of prey these ecosystems provide for growth and survival (Nagelkerken et al. 2001; Mateo et al. 2010). For example, juvenile and sub adult Lemon sharks (*Negaprion brevirostris*) and Barracuda (*Sphyraena barracuda*) almost exclusively use mangrove and back reef ecosystems as feeding grounds, as these species are particularly vulnerable to predation by larger apex predators prior to their ontogenetic shift to the reef environment (Nagelkerken et al. 2001; Ellis & Bell 2004). Therefore, given the presence of predators in mangrove ecosystems, shelter is an important but potentially limiting resource for fishes (reviewed by Hixon 1991) as it establishes the only barrier between predator and prey in mangroves (Forrester & Steele 2004).

Previous research has investigated the impact of lionfish on shelter use in a native coral reef species. Raymond et al. (2015) showed that Nassau grouper

in the presence of lionfish display two avoidance behaviours. When Nassau grouper interact with much smaller lionfish they actively avoid the lionfish by sheltering slightly less (Raymond et al. 2015). However, when the lionfish and grouper are of similar size, grouper avoid the lionfish by actively increasing shelter use and avoiding the space in proximity to the lionfish (Raymond et al. 2015). This result suggests that grouper and lionfish are likely not competing for space but are displaying avoidance behaviour out of fear of being eaten (Raymond et al. 2015). Additionally, the proportion analysis of this data did not indicate lionfish dominance, despite lionfish indicating no difference in behaviour. However, no studies have investigated these relationships when salinity of the surrounding environment fluctuates daily, such as in some mangrove ecosystems. Because of the strong but predictable influence of tides on some mangrove and back reef ecosystems, these ecosystems can be considered self-regulating to overexploitation by native marine predators (Laegdsgaard & Johnson 2001). As the tide leaves and moves seaward, the surrounding environment becomes shallow, water temperature increases and depending on location may become hypo or hyper saline, which may cause some predators to leave (Diele & Simith 2006). This may in turn restrict the amount of time that predators have access to prey in mangrove ecosystems (Ellis & Bell 2004).

Recently, lionfish have been shown to tolerate a wider range of salinity than previously thought. Jud et al. (2015) were among the first to report not only the presence of lionfish as far as 6.6 km from the ocean into the Loxahatchee River estuary (Florida, USA) but also the ability of lionfish to tolerate low salinity with 15 of 16 individuals surviving in excess of 28 days in a salinity treatment of only 7 ppt. In Chapter 2 of this thesis, lionfish were reared in aquaria under differing salinity treatments (low / 10 ppt, mid / 20 ppt and control / 37 ppt), similar

conditions to those lionfish might be exposed to moving from a coral reef to a mangrove ecosystem to investigate the effect of hyposalinity on growth, metabolic rate, maximum food consumption, feeding rates, and specific dynamic action. Results showed that lionfish were able to survive in hyposaline conditions for over two months, but aspects of their feeding, aerobic capacity and digestion were reduced.

The vast majority of fish species are specialised to either hyper or hypo-osmoregulate but a limited ability to do both, suggesting limited dispersal across both marine and freshwater ecosystems (Evans 1984). In fish species that are able to move across marine and freshwater ecosystems it can take between 3 - 12 days to acclimate, with the process being costly and sometimes fatal (Ferraris et al. 1988; Nonnotte & Truchot, 1990; Jensen et al. 2002; Sampaio & Bianchini 2002). However, certain species have adapted to live in variable habitats and migrate throughout their life cycle, thus exposing themselves to habitats of different salinity. The success of a fish in variable salinity habitats depends on the speed in which they can acclimate and their ability to maintain biological function during these periods. It has also been suggested that there is plasticity within a species to both hyper and hypo-osmoregulate depending on origin habitat salinity (Christensen et al. 2019).

Significant lionfish mortality has been shown to occur in salinities below 7 ppt (Jud 2011) suggesting that lionfish indeed have a salinity threshold. Relatively few species have a broad capacity to maintain constant body fluid and ion composition within both dilute and concentrated environments due to the expensive process of ion loss and diffusion (Serrano 2008). This has not been directly tested with lionfish, however due to their native range and typical association with reef habitats it is assumed for this study that lionfish are

osmoregulators, having to actively pump ions when in more saline water to combat water loss (Marshall & Grosell 2005). Therefore, if the behavioural and physiological abilities of lionfish shown in the marine environment remain similar under hyposaline conditions, the homeostasis of the biota found in mangrove and back reef ecosystems may be threatened. Lionfish may not only increase predation on juvenile and mangrove specialist species directly, but may also out-compete native species for food and shelter, potentially leading to increased predation by apex predators such as juvenile sharks (Ellis & Bell 2004).

Juvenile Schoolmaster snapper (*Lutjanus apodus;* hereafter referred to simply as snapper) are a common tropical fish species frequently found in shallow mangrove and back reef ecosystems (Laegdsgaard & Johnson 2001; Luo et al. 2009; Mateo et al. 2010). Given their high abundance they are also a food source for a variety of marine predators (MacDonald et al. 2009; Mateo et al. 2010; Serafy et al. 2015). Snapper appear to target similar prey to lionfish - small fish and crustaceans (Morris & Akins 2009; Côté & Maljković 2010; Jud et al. 2011; Layman & Allgeier 2012) - potentially indicating that they may compete with lionfish for shelter and food in mangroves and back reef ecosystems, although this has yet to be explored.

Using a habitat competition experiment in the laboratory at two salinity treatments (low / 10 ppt and control / 37 ppt) the objectives of the present study were; 1) investigate the time spent in shelter by lionfish and snapper separately and together in an experimental arena, 2) determine whether lionfish could out-compete snapper for shelter and 3) identify key behaviours in both lionfish and snapper and determine how these are affected by salinity. Because of the potential for reduced physiological activity of lionfish, such as longer digestion, in hyposaline conditions, as shown in Chapter 2, the present study predicted that

lionfish would out-compete snapper for shelter under ambient conditions (37 ppt), but would show reduced competition in low salinity (10 ppt). If disruptions to osmotic balance act upon marine fish in a similar manner to disruptions to pH balance (eg. decreases in olfactory responses to detect predators), it was predicted that snapper will exhibit less fear and be more inquisitive towards the lionfish in the 10 ppt treatment, than the 37 ppt treatment.

Methods

Animal capture and transport

Lionfish were collected by divers on SCUBA using clear plastic hand nets from patch reefs (~ 3 m depth) located adjacent to the Cape Eleuthera Institute (CEI), The Bahamas (24°50′ N; 76°20′ W). Patch reefs were located using a handheld GPS (Garmin eTrek, Kansas, USA) and known coordinates from a previous lionfish study conducted at CEI (Smith et al. 2017). Snapper were collected from Paige creek, Eleuthera, The Bahamas, using conical funnel traps, baited with pieces of blue crab and shrimp. All snapper collected were of a similar small size to emulate those most likely found in mangrove ecosystems (juveniles) and because we assumed that this size is most likely to be impacted by lionfish. Both species were transported in large coolers filled with seawater to aquarium holding facilities at CEI. An air stone was placed in the coolers to ensure adequate air saturation during transport.

Animal rearing, husbandry and feeding

Lionfish (average length 14.38 cm ± 0.13 SE, n = 12) and snapper (average length 7.92 cm ± 0.145 SE, n = 12) were housed separately, and equally divided across two aquaria (lionfish in a circular aquarium: 160 cm diameter X 60 cm depth and snapper in a rectangular aquarium 242 cm X 58 cm X 30 cm). All

aquaria were supplied with flow through seawater at ambient temperature (mean 25.0 °C ± 2.7), salinity (mean 37.0 ppt ± 2.3) and photoperiod. All fish were fed *ad libitum* daily. Lionfish were fed live Atlantic silversides and the snapper were fed pieces of defrosted shrimp. Aquaria temperature, salinity and water flow were checked daily and the aquaria cleaned using a vacuum siphon and scrub brush to remove any debris accumulating on the bottom. All fish were left for one week after capture to allow for settling and recovery. All fish were in good external condition prior to experiments.

Salinity treatments

To investigate the effect of salinity on the habitat use of lionfish and snapper, salinity of one lionfish aquarium and one snapper aquarium were lowered by 5 ppt every 24 hours (Jud et al. 2015) by the addition of fresh water until it reached 10 ppt, similar to what may be expected during ebb tides in mangrove ecosystems. Adequate mixing was achieved using an air stone and by ensuring the fresh water intake was located on the bottom of the aquarium. Water changes were conducted at 09:00 daily with a continuous feed of fresh and salt water for 1.5 hours. Fish were left for one month to acclimate to the hyposaline condition. Salinity levels were checked daily using a refractometer.

Behavioural interactions

To test whether lionfish are likely to displace native snappers in mangrove ecosystems, habitat use of lionfish and snapper was quantified in isolation and together in an experimental arena (86 cm wide × 258 cm long × 43 cm deep), which included an open area and shelter (a single red mangrove root (Fig.3.1, Fig. 3.2)). Fish were randomly selected to take part in each trial. All snapper were of similar size. Lionfish varied in size, but were never smaller than snapper. To quantify the location and movements of each fish during the trial, the arena was

divided into 30 equally sized square segments using a printed grid. A camera (GoPro Hero Black, Go Pro Ltd, USA) was suspended above the tank to film the field of view. An opaque removable partition divided the arena into two areas to allow for both an isolation period (where the snapper or lionfish each had access to the shelter individually; Fig. 3.1A and C, respectively) and an interaction period (where the lionfish and snapper competed for access to the shelter; Fig. 3.1B).

Each trial consisted of a 24-hour settling period, where both lionfish and snapper where placed in the experimental tank with the divide in place to allow the individual fish to become comfortable with the space, and three experimental periods. 1) a 90-minute isolation period (where either the snapper or lionfish solely had access to the shelter), 2) a 90-minute interaction period (where both the lionfish and snapper shared the tank and competed for access to the shelter) and finally 3) another 90-minute isolation period (where either the lionfish or snapper solely had access to the shelter). To avoid any potential confounding effect of time, all trials were conducted between 13:00 and 17:30 daily. Each fish was only used once in the experiment to remove any potential effects of learning.

Following each trial, the experimental arena was emptied, cleaned and filled with water at the same temperature and salinity to the relevant holding aquarium.

Video analysis

All videos were analysed by a single researcher (RT) to avoid observer bias. Using the grid on the bottom of the arena as a reference, the video was paused every two minutes and the position of each individual fish was noted (Fig. 3.2). This provided a total of 45 positions for each individual for each of the three 90-minute periods. From these values, the total time spent by each individual in the mangrove shelter was calculated for both the isolation and interaction periods.

An individual was deemed as being in the shelter if 50% of the body was under the cover of the mangrove.

Behavioural ethogram

Each interaction trial, from both treatments (n total trials = 12), was viewed continually for the 90-minute period and a behavioural ethogram completed. Key behaviours were identified using previous literature noting aggressive and passive behaviours in lionfish (Cure et al. 2012; Black et al. 2014; Table 3.1).

Calculations and statistical analyses

The effects of salinity treatment (low / 10 ppt or control / 37 ppt), species (lionfish or snapper) and period (isolation or interaction) on shelter use were evaluated using a linear mixed effect model and where appropriate included all two-way interactions. Salinity treatment, species (lionfish and snapper) and period were included as fixed effects. To control for any confounding effect of experiment order (i.e. order of interaction and isolation period) we included order as a random effect. Models were compared using AIC criterion before and after removal of interaction terms and independent variables that were not significant at $p < 0.05$. The effect of salinity treatment (low / 10 ppt or control / 37 ppt) on behaviours identified and defined in the ethogram (Table 3.1) were compared using a t-test. All analyses were conducted using RStudio Desktop 1.2.1335 – © 2019, Inc. statistical software (RStudio Team 2019).

Ethics

All work was carried out under the Bahamas Department of Marine Resources permit number MAMR/FIS/17 and reviewed by the University of Exeter (2017/1760). All snapper were returned to their site of capture after the experiment. However, as lionfish are an invasive species in the Atlantic and wider Caribbean (Whitfield & Hare 2003), they could not be released back to the wild

after experiments, and were instead euthanised using a lethal solution of water and clove oil. This is a widely accepted method of fish euthanasia as suggested by The Guide for the Care and Use of Laboratory Animals (NRC 2010).

Results

Salinity did not affect the amount of time the lionfish or snapper spent in shelter during the isolation periods (when a lionfish or snapper had unchallenged access to the shelter) and interaction periods (when both the lionfish and snapper interacting for access to the shelter) ($F_{1, 10} = 3.15$, $p = 0.10$, Fig. 3.3). However, there was a significant difference in shelter use of snapper when interacting with lionfish in both the 37 ppt ($F_{1, 10} = 20.36$, $p < 0.01$, Fig. 3.3) and 10 ppt ($F_{1, 10} = 11.79$, $p < 0.01$, Fig. 3.3) treatments. Overall, snapper spent 53% less time in the shelter when in the presence of lionfish, regardless of salinity. Interestingly, there was no difference in shelter use of lionfish whether in absence or presence of a snapper across either salinity treatment suggesting a highly asymmetrical interaction (37 ppt; $F_{1, 10} = 0.16$, $p = 0.70$ and 10 ppt; $F_{1, 10} = 0.64$, $p = 0.44$, Fig. 3.3).

Seven key behaviours were identified from the ethogram: cornering, chasing, charging and fin display were grouped as aggressive behaviours displayed by the lionfish. Stand-off was described as an inquisitive behaviour displayed by the snapper. Displacement and avoidance were grouped as fear behaviours displayed by the lionfish (Table 3.1). There was no significant difference in any of the behaviours of interest between the 10 ppt and 37 ppt (control) treatments (Table 3.2, Fig. 3.4).

On average, snapper approached the lionfish on 7 occasions in the 37 ppt treatment and on 3 occasions in the 10 ppt treatment (Table 3.2), with the

maximum occurrence being 15 counts in one of the 37 ppt trials. Furthermore, this trial also showed the highest count of displacement for snapper across both salinity treatments.

Discussion

Many economically important fish species targeted by recreational and commercial fisheries have parts of their life cycle that depend upon shelter in mangroves (Nagelkerken et al. 2001; Ellis & Bell 2004; Faunce & Serafy 2006; Mateo et al. 2010). Previous studies have suggested that lionfish have the ability to invade and survive in mangrove and back reef habitats (Barbour 2010; Jud et al. 2011, Jud et al. 2012; Prakash et al. 2012; Kulbicki et al. 2012; Jud & Layman 2012; Claydon et al. 2012; Pimiento et al. 2015). In the present study, lionfish were not only able to survive in low salinity but displaced native snapper from the shelter. The fact that lionfish occupied the mangrove shelter regardless of the presence or absence of the snapper indicates that the invader is a superior competitor for refuge space. Given the importance of mangrove ecosystems for a wide range of fish species, the movement of invasive lionfish into these ecosystems is of concern (Jud et al. 2015). If other mangrove fish species show similar behavioural changes as the snapper in the present study, and lionfish cause significant displacement thus leading to increased predation, the commercial, recreational and ecological impacts could be significant.

Lionfish, on invaded coral reef habitats in the Caribbean, have been shown to reduce fish and invertebrate abundance by 79% through unrestricted predation (Albins & Hixon 2008; Albins 2015) and to cause extirpations of native reef fishes (Albins 2015; Ingeman 2016). Acting as opportunistic predators, lionfish have broad diets and have been shown to feed on over 40 species of small-bodied reef

fishes, representing over 50% of common reef species (Morris & Akins 2009; Green et al. 2012; Côté et al. 2013). Mangrove ecosystems are not only rich in species diversity and abundance, but provide critical nursery habitat for a range of reef fish (Laegdsgaard & Johnson 2001; Nagelkerken et al. 2001; Mumby et al. 2004; Faunce & Serafy 2006; Nagelkerken et al. 2008; Mateo et al. 2010), such that predation by lionfish in mangroves may have catastrophic impacts on the population numbers of small fishes, and ultimately the recruitment of native fish species to reefs (Robertson & Duke 1987; Nagelkerken et al. 2001). Lionfish in the present study did not attempt to feed on snapper, but it is suspected this is more likely a result of gape limitation, rather than an avoidance of them as prey (Green et al. 2019). Lionfish were consistently larger than all the snapper but not large enough to consume snapper. In contrast to Raymond et al. (2015), lionfish and snapper were not size matched, but rather the snapper were selected to represent the typical size found in mangrove ecosystems, that would likely be encountered by lionfish. However, similar to Raymond et al. (2015) the results of this experiment identified snapper actively fleeing the shelter of the mangrove as the lionfish approached, despite being apparently naïve to the species. Alternatively, Raymond et al. (2015) found that grouper use the shelter significantly more when the two competing species are of similar size. It could be argued that this effect did not occur in the present study because the lionfish was consistently larger than the snapper. Alternatively, it may be that the snapper simply display a different avoidance behaviour towards lionfish compared to grouper, suggesting context dependency.

In addition to mangrove and back reef ecosystems being important for recreational and commercial fish and invertebrate species, they also act as critical foraging habitats for many marine predators (Wolff et al. 2000; Laegdsgaard &

Johnson 2001; Ellis & Bell 2004). If the reduced shelter use by the snapper in the presence of lionfish observed in the present study, occurs in the wild, snapper could be subject to increased secondary predation by Lemon sharks (*Negaprion brevirostris*) and Great Barracuda (*Sphyraena barracuda*) (Wolff et al. 2000; Laegdsgaard & Johnson 2001; Ellis & Bell 2004; Fig. 3.5). In turn, this could reduce recruitment of snapper onto reefs, and ultimately the overall abundance of snapper (Laegdsgaard & Johnson 2001).

The results of the behavioural ethogram provided insight into the steps leading to the displacement of snapper from shelter. Snapper were not solely displaced from the mangrove shelter, but rather consistently avoided the lionfish whether under the mangrove shelter or in the open, perhaps suggesting some form of learning to avoid lionfish during the study or from previous encounters in the wild. Additionally, because snapper continued to move away from lionfish and shelter, they ultimately swam further into the open. During the experiment, the mangrove root shelter was placed on one end of the tank to allow for open space to simulate the open water found in mangrove ecosystems. When lionfish moved into the mangrove shelter, most snapper swam to the far end of the arena, suggesting that snapper may swim into the open water in a natural mangrove ecosystem, although testing this would be a worthwhile area for future studies.

In all trials, lionfish exhibited aggressive behaviours such as fin displays, chasing and charging. However, there was no difference in the relative occurrence of these behaviours between the two salinities, suggesting that they were not influenced by changes in salinity (Careau et al. 2008; Biro & Post 2008; Copeland et al. 2011; Martins et al. 2011; Rupia et al. 2016). Although snapper appeared to avoid lionfish, every snapper, across all trials approached and examined the lionfish, potentially because the snapper are naïve to lionfish

(Anton et al. 2016). The highest count of inspection of a lionfish by a snapper also occurred with the highest count of displacement.

Salinity had no effect on shelter use of either species. Snapper were predicted to be well adapted to varying salinity due to their ability to move between reef and mangrove ecosystems freely for foraging and protection (Luo et al. 2009). However, because lionfish are typically considered a marine reef fish, it was predicted that lionfish activity would be reduced in hyposaline conditions due to the energetic cost of moderating ion exchange (Chapter 2), and may spend more time resting and have a reduced ability to out-compete snapper. In their invaded range, lionfish have relatively few predators (Mumby et al. 2011; Hackerott et al. 2013), which allows them to feed uninhibitedly regardless of the need of shelter for protection. Results from Chapter 2 suggested that lionfish have a lower maximum metabolic rate in lower hyposaline conditions. Therefore, the present study hypothesised two expected outcomes; 1) lionfish in the 10 ppt treatment would be slower, with less capacity to out-compete snapper for shelter, therefore snapper displacements would be lower and 2) lionfish because of reduced activity would use shelter more frequently, as their abilities to flee become inhibited. The findings of this experiment saw no difference in snapper displacement between the two salinities, suggesting that salinity had no effect on the shelter use and potential competitive nature of the lionfish. Lionfish continued to use the shelter (60%) regardless of salinity or snapper presence, suggesting that the lionfish is a superior competitor for refuge space.

The results of the present study provide an insight into the potential negative impacts of lionfish invading mangrove ecosystems. As lionfish were able to survive low salinity, they could feed regardless of tides and changes in salinity, ultimately altering food web structures in these ecosystems (Fig. 3.5). Blood

profiling of individual lionfish (Greenwood et al. 2008; Cooke et al. 2008) would allow identification of how their behaviours and physiological processes change as they move through environments of differing salinities. This would be similar to the work of Chapter 2 but using this information in addition to behavioural trials, would allow further replication of the fluctuating environment of a mangrove ecosystem.

In order to determine the ecological significance of the displacement of native species caused by lionfish invading mangroves, additional experiments could investigate how snapper react with multiple native species before they have interacted with lionfish. Also, as schoolmaster snapper are a schooling fish, it would be beneficial to investigate how snappers react when there are multiple individuals to see whether the effect of lionfish on shelter use is the same. Finally, altering the size and number of shelters would allow a further insight into how the shelter is used and whether size and density influences whether a snapper uses a shelter in the presence of a lionfish.

The expansion of non-native species are both a regional and global concern to biodiversity and an over-looked by-product of global change (Vitousek et al. 1996; Chapin et al. 1998; Mack et al. 2000). Researchers have continued to investigate the relative competitive performance of native versus invasive species and the differences in competitive abilities between them, which could result in population declines of native species (Daehler 2003). Lionfish have been shown to outcompete native small grouper (Albins 2013) but not large native grouper (Ellis & Faletti 2016). Numerous studies have shown the importance of predators in shaping the structure of fish communities within marine ecosystems. However, these can be easily disrupted by invasive species, such as the lionfish. Albins (2013) identified that the lionfish had a stronger ecological effect on native

coral-reef fish populations causing an 83.7% reduction compared to native groupers (36.3% reduction) over an 8-week period. They concluded that lionfish may pose a substantial threat to native coral-reef fish communities. However, Ellis & Faletti (2016) found that juvenile reef fish abundance did not differ when both lionfish and red grouper were present, highlighting that native predators may in fact improve the negative effects of lionfish. These findings, similar to the work of Raymond et al. (2015), indicate the potential effect of size variation on the extent of the negative impacts of the lionfish. Together with the findings of the present study, these publications identify the potential ecological impact of lionfish on reef fish biodiversity.

The results of the present study suggest that lionfish may have the capacity to displace native fish from shelter, which may have an insidious effect on trophic dynamics of both mangrove and reef ecosystems they serve as nurseries for (Laegdsgaard & Johnson 2001; Mateo et al. 2010).

Figures and Tables

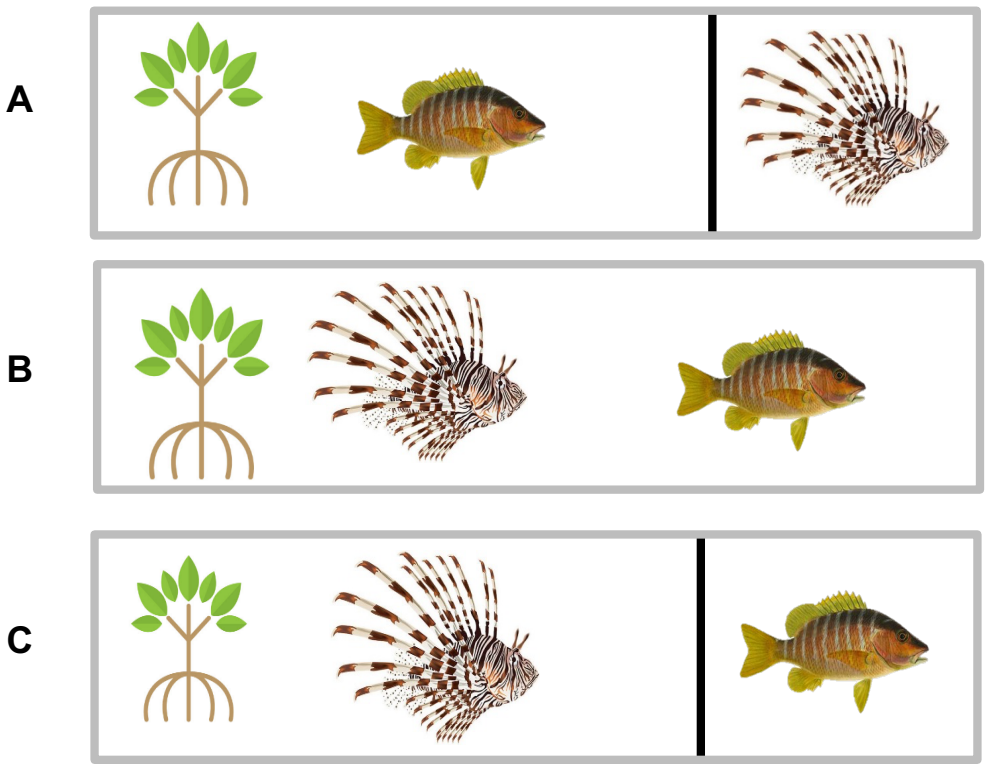


Figure 3.1. A schematic showing the three experimental stages filmed for each behavioural trial. **(A)** an isolation period in which only the Schoolmaster snapper had access to the shelter, **(B)** an interaction period in which the Schoolmaster snapper and lionfish both had access to the shelter and **(C)** an isolation period in which only the lionfish had access to the shelter. All fish were given 24 hours to acclimatise to the experimental arena, with each experimental stage filmed for 90 minutes. Each trial was conducted in the relevant salinity in which the subjects had been acclimated for 1 month.

Mangrove image sourced from http://freevectorfinder.com/free-vectors/mangrove/

Snapper image sourced from https://designbundles.net/enliven-designs/121134-fish-vintage-schoolmaster

Lionfish image sourced from https://www.kisspng.com/png-red-lionfish-invasive-species-clip-art-lionfish-2687012/

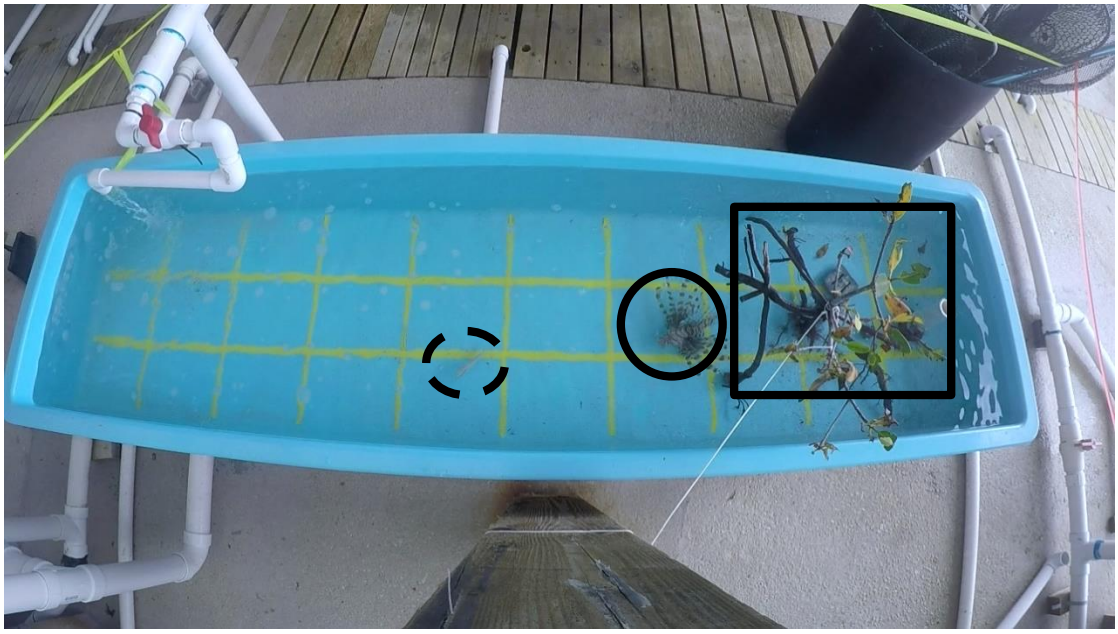


Figure 3.2. Top down photograph of the experimental arena used during the interaction stage of the experiment. The arena was split into a grid system to allow for positions of fish to be determined. The lionfish is shown by the solid circle, the Schoolmaster snapper by the dashed circle and the mangrove (shelter) by the square.

Photograph taken as a still from video, taken by researcher (RT).

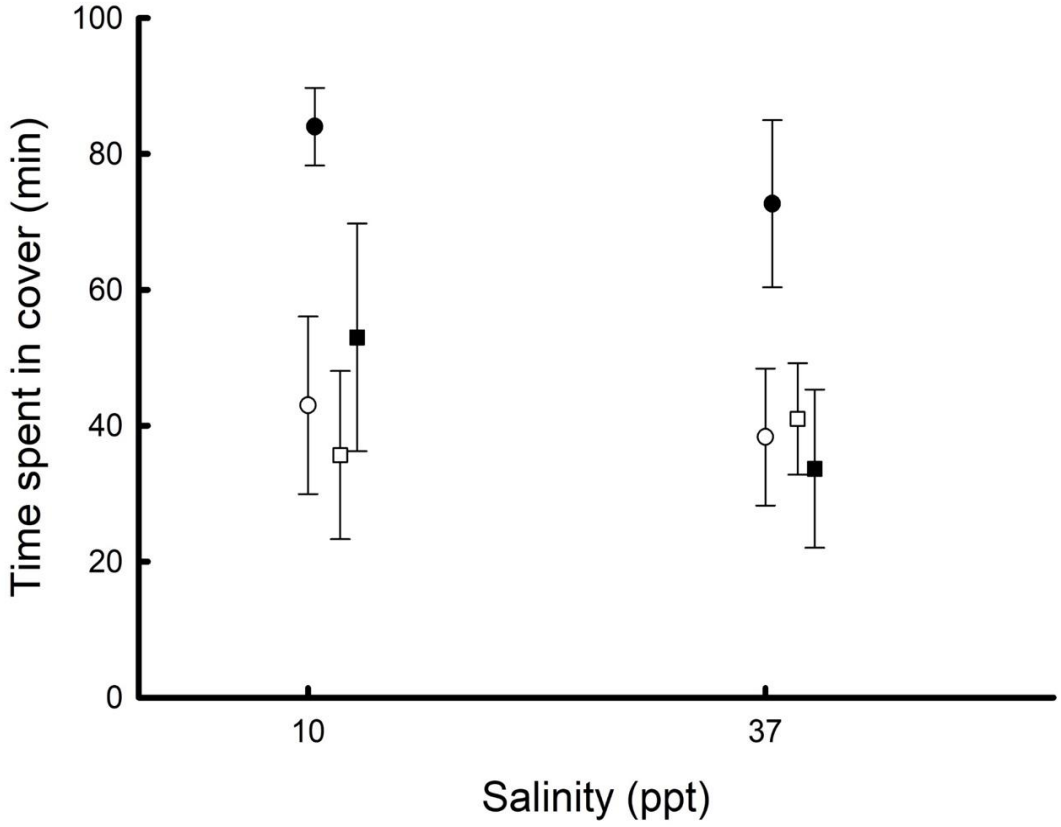


Figure 3.3. The relationship between mean time spent in the cover of a mangrove (min.) and salinity (low / 10 ppt and control / 37 ppt) for Schoolmaster snapper (black symbols) and lionfish (white symbols) for both an isolation period (circles), where the snapper or lionfish only had access to the mangrove shelter and an interaction period (squares), where the snapper and lionfish both had access to the mangrove shelter. Error bars represent 95% CI.

NB: The significant decrease in shelter use of the Schoolmaster snapper in both salinity treatments (10 ppt and 37 ppt) when competing for shelter with lionfish.

Behaviour	Description
Displacement [(a)]	The snapper moves out of the cover of the mangrove as the lionfish moves in
Avoidance [(a)]	The snapper moves away from the lionfish regardless of being in or out of the mangrove
Stand-off [(b)]	The lionfish and snapper face each other without moving
Cornering [(c)]	The lionfish forces the snapper into a corner of the arena
Chasing [(c)]	The lionfish rapidly swims after snapper
Charging [(c)]	The lionfish darts towards the snapper
Display [(c)]	The lionfish moves towards the snapper with fins flared

Table 3.1 A comprehensive description of behaviours included in the behavioural ethogram and defined from previous research on aggressive behaviours displayed by lionfish and passive behaviours displayed by snapper. (a) denotes fear behaviours displayed by the snapper, (b) denotes inquisitive behaviours displayed by the snapper towards the lionfish and (c) denotes aggressive behaviours displayed by the lionfish towards the snapper.

Behaviour	37 ppt	10 ppt	T-test Result
Displacement	56.0 ± 4.2	47.8 ± 2.1	t = 1.7, df = 7.3, p = 0.10
Avoidance	50.0 ± 10.4	32.2 ± 0.8	t = 1.7, df = 5.1, p = 0.10
Stand-off	2.0 ± 0.7	1.8 ± 0.7	t = 0.2, df= 1.0, p = 0.90
Cornering	5.5 ± 1.7	3.8 ± 1.6	t =0.7, df = 9.9, p = 0.49
Chasing	8.8 ± 3.3	3.5 ± 0.9	t = 1.5, df = 5.8, p = 0.18
Charging	3.5 ± 1.9	2.5 ± 1.1	t = 0.4, df = 8.0, p = 0.67
Display	18.2 ± 5.8	10.2 ± 1.0	t = 1.4, df = 5.2, p = 0.23

Table 3.2 Experiment values for behaviours displayed by lionfish and snapper during interaction trials and when acclimated to two salinity treatments (low / 10 ppt and control / 37 ppt). Values represent the mean ± SE.

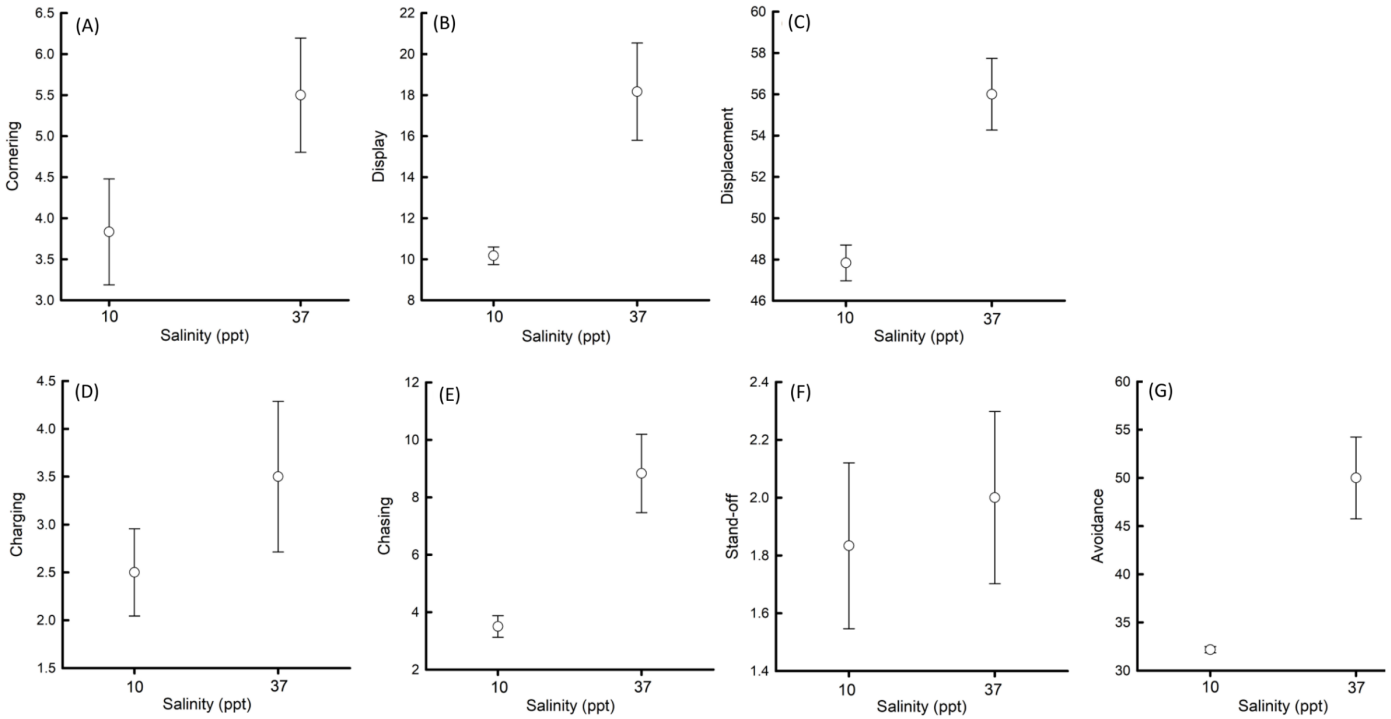


Figure 3.4 The relationship between average count of each behaviour and salinity (low / 10 ppt and control / 37 ppt) during the interaction period, when snapper and lionfish both had access to the mangrove shelter (n = 12). Error bars represent ± SE.

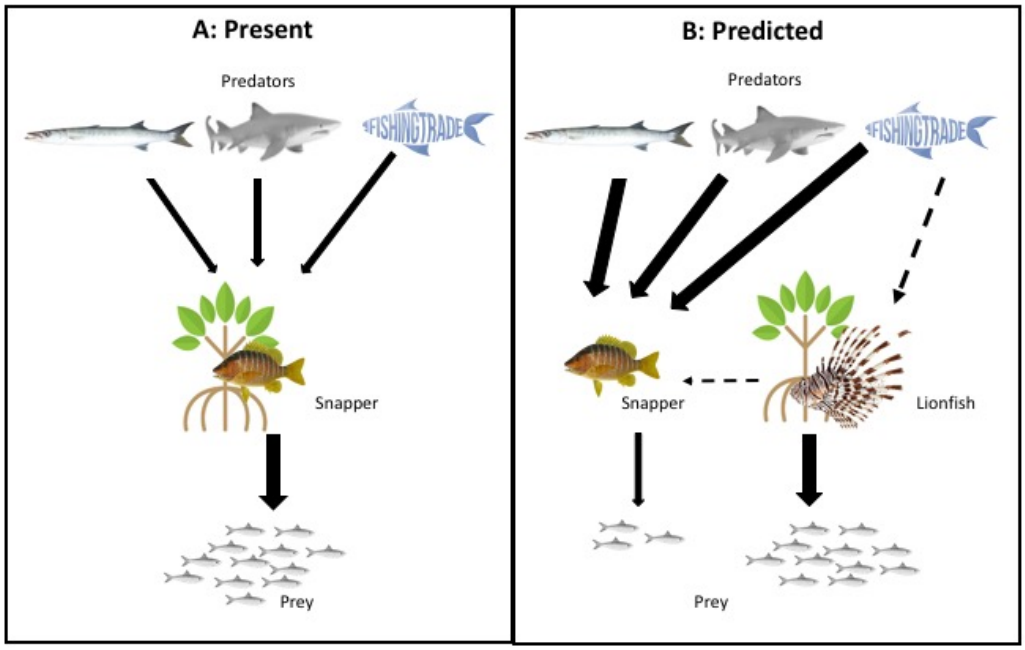


Figure 3.5 A schematic diagram showing; **(A)** the current predicted food chain within a mangrove ecosystem, before the introduction of lionfish and **(B)** the potential trophic cascades as a result of the introduction of lionfish into a mangrove ecosystem. The introduction of lionfish, as suggested by the present study, could cause snapper to move into the open and cause increased predation. In turn this could cause a reduction in predator numbers and a decrease in fishing trade due to a decrease in snapper population size from increased predation. The dotted lines represent a predicted change in fishing trade as there is now an increasing market for lionfish and also that lionfish may begin to prey on small snapper if prey supplies become limited.

Barracuda image sourced from https://www.pinterest.com.au/pin/347129083762588272/
Lemon shark image sourced from https://store.safariltd.com/products/lemon-shark
Fishing trade image sourced from https://fishing-trade.com/
Mangrove image sourced from http://freevectorfinder.com/free-vectors/mangrove/
Snapper image sourced from https://designbundles.net/enliven-designs/121134-fish-vintage-schoolmaster
Lionfish image sourced from https://www.kisspng.com/png-red-lionfish-invasive-species-clip-art-lionfish-2687012/
Prey fish image sourced from https://www.graytaxidermy.com/baitfish-gallery.html

Chapter 4: General Discussion

The invasion of lionfish in the Western Atlantic and wider Caribbean is one of the most documented marine invasions to date (Sutherland et al. 2010). However, research has primarily focused on reef environments with minimal salinity fluctuations (35 - 39 ppt). In recent years lionfish have been documented in mangrove ecosystems (Barbour et al. 2010; Jud et al. 2011; Jud & Layman 2012; Prakash et al. 2012; Kulbicki et al. 2012; Claydon et al. 2012; Pimiento et al. 2015) resulting in an expansion of experimental techniques (Table 1.1). Mangrove habitats face a number of threats, both anthropogenic and natural, including overfishing (Giuliani et al. 2004), pollution (Satheeshkumar et al. 2012), habitat destruction (Alongi 2002) and now invasive species (Gilman et al. 2008). However, mangroves offer an abundance of valuable ecosystem services such as coastal protection, water filtration and fish nursery habitats (Primavera 1998; Barbier 2006; Barbier et al. 2011; Sandilyan & Kathiresan 2015). Due to the voracious nature of the lionfish, their presence in these critically important nursery habitats is concerning for the future recruitment of native species (Mateo et al. 2010; Albins & Hixon 2013).

In **Chapter 2,** I establish through growth, feeding and metabolic analyses the lionfish's ability to survive in a low salinity habitat at two different food levels. I identified subtle changes in the lionfish's growth, feeding rates, SMR, SDA_{Max}, SDA_{Scope} and SDA_{Total} at low food availability. However, I did find a reduction in MMR, AS and SDA duration in the low salinity treatment, indicating a potential for physiological impairment in aerobic capacity at lower salinities. When these factors are combined, the reduction in MMR and AS may be due to longer digestion, lower maximum food consumption, and decreased growth rate in the high food availability experiment. Given the ability of lionfish to tolerate low

salinity, updated range expansion models should incorporate back reef ecosystems to improve accuracy of predicted range expansion.

In **Chapter 3,** I used behavioural trials and video analysis to identify the effect of lionfish on shelter use of a native species, Schoolmaster snapper. Here I found that lionfish caused snapper to be displaced from shelter and protection of the mangrove root in the presence of lionfish (over 50% occurrence), irrespective of salinity, suggesting an increased risk of predation from other mangrove meso-predators following the invasion of lionfish. Through more detailed analysis using a behavioural ethogram, I provided further insights into the steps of displacement. Interestingly, the results of the behavioural ethogram identified that snapper did not remain outside of the mangrove once displaced, but rather continually moved in and out of the mangrove, actively avoiding the lionfish. I also identified that there is no difference in behaviours of either the lionfish and the snapper in the two salinities tested, despite the predictions based on findings from Chapter 2.

The movement of lionfish into mangrove ecosystems remains understudied. Therefore, the results of Chapters 2 and 3 contributes to the knowledge of the species' ability to survive and thrive in these ecosystems and could be used to further the predicted range expansion of the species. Together the results of these chapters develop an understanding of how lionfish are surviving in these habitats, how they may impact native species, the potential progression that may occur if lionfish continue to invade mangrove ecosystems and resulting trophic shifts. Although the continual impact of the invasion seems to have somewhat plateaued (Benkwitt et al. 2017), further research must include brackish waters in their predictions and be incorporated into a range of approaches such as physiology and behaviour when developing future range

expansion models (Betancur et al. 2011; Bernal et al. 2015; Evangelista et al. 2016; Grieve et al. 2016).

Bibliography

Albins, M.A., 2013. Effects of invasive Pacific red lionfish Pterois volitans versus a native predator on Bahamian coral-reef fish communities. *Biological Invasions*, 15(1), pp.29-43.

Albins, M.A., 2015. Invasive Pacific lionfish Pterois volitans reduce abundance and species richness of native Bahamian coral-reef fishes. *Marine Ecology Progress Series*, 522, pp.231-243.

Albins, M.A. and Hixon, M.A., 2008. Invasive Indo-Pacific lionfish Pterois volitans reduce recruitment of Atlantic coral-reef fishes. *Marine Ecology Progress Series*, 367, pp.233–238.

Albins, M.A. and Hixon, M.A., 2013. Worst case scenario: potential long-term effects of invasive predatory lionfish (Pterois volitans) on Atlantic and Caribbean coral-reef communities. *Environmental Biology of Fishes*, 96(10–11), pp.1151–1157.

Alongi, D.M., 2002. Present state and future of the world's mangrove forests. *Environmental conservation*, 29(3), pp.331–349.

Alsop, D. and Wood, C., 1997. The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (Oncorhynchus mykiss). *Journal of Experimental Biology*, 200(17), pp.2337–2346.

Anton, A., Cure, K., Layman, C.A., Puntila, R., Simpson, M.S. and Bruno, J.F., 2016. Prey naiveté to invasive lionfish Pterois volitans on Caribbean coral reefs. *Marine Ecology Progress Series*, 544, pp.257-269.

Arias-González, J.E., González-Gándara, C., Cabrera, J.L. and Christensen, V., 2011. Predicted impact of the invasive lionfish Pterois volitans on the food web of a Caribbean coral reef. *Environmental research*, 111(7), pp.917-

925.

Azzurro, E. and Bariche, M., 2017. Local knowledge and awareness on the incipient lionfish invasion in the eastern Mediterranean Sea. *Marine and Freshwater Research*, 68(10), pp.1950-1954.

Baltz, D.M., 1991. Introduced fishes in marine systems and inland seas. *Biological Conservation*, 56(2), pp.151–177.

Barbier, E.B., 2006. Natural barriers to natural disasters: replanting mangroves after the tsunami. *Frontiers in Ecology and the Environment*, 4(3), pp.124–131.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C. and Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. *Ecological monographs*, 81(2), pp.169-193.

Barbour, A.B., Montgomery, M.L., Adamson, A.A., Díaz-Ferguson, E. and Silliman, B.R., 2010. Mangrove use by the invasive lionfish Pterois volitans. *Marine Ecology Progress Series*, 401, pp.291-294.

Bariche, M., Torres, M. and Azzurro, E., 2013. The presence of the invasive Lionfish Pterois miles in the Mediterranean Sea. *Mediterranean Marine Science*, 14(2), pp.292-294.

Bax, N., Williamson, A., Aguero, M., Gonzalez, E. and Geeves, W., 2003. Marine invasive alien species: a threat to global biodiversity. *Marine policy*, 27(4), pp.313-323.

Benkwitt, C.E., Albins, M.A., Buch, K.L., Ingeman, K.E., Kindinger, T.L., Pusack, T.J., Stallings, C.D. and Hixon, M.A., 2017. Is the lionfish invasion waning? Evidence from The Bahamas. *Coral Reefs*, 36(4), pp.1255-1261.

Bernal, N.A., DeAngelis, D.L., Schofield, P.J. and Sealey, K.S., 2015. Predicting spatial and temporal distribution of Indo-Pacific lionfish (Pterois volitans) in

Biscayne Bay through habitat suitability modeling. *Biological Invasions*, 17(6), pp.1603-1614.

Betancur, R., Hines, A., Acero, A., Ortí, G., Wilbur, A.E. and Freshwater, D.W., 2011. Reconstructing the lionfish invasion: insights into Greater Caribbean biogeography. *Journal of Biogeography*, 38(7), pp.1281-1293.

Biggs, C.R. and Olden, J.D., 2011. Multi-scale habitat occupancy of invasive lionfish (Pterois volitans) in coral reef environments of Roatan, Honduras. *Aquatic Invasions*, 6(3), pp.447-453.

Biro, P.A. and Post, J.R., 2008. Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. *Proceedings of the National Academy of Sciences*, 105(8), pp.2919–2922.

Black, A.N., Weimann, S.R., Imhoff, V.E., Richter, M.L. and Itzkowitz, M., 2014. A differential prey response to invasive lionfish, Pterois volitans: prey naiveté and risk-sensitive courtship. *Journal of Experimental Marine Biology and Ecology*, 460, pp.1-7.

Blaustein, R. J. (2001). “Kudzu's invasion into Southern United States life and culture,” in *The Great Reshuffling: Human Dimensions of Invasive Species*, ed. J. A. McNeeley (Gland; Cambridge: IUCN, The World Conservation Union), 55–62.

Boeuf, G. and Payan, P., 2001. How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 130(4), pp.411–423.

Brown, C., Jones, F. and Braithwaite, V., 2005. In situ examination of boldness–shyness traits in the tropical poeciliid, Brachyraphis episcopi. *Animal Behaviour*, 70(5), pp.1003–1009.

Burke, M.J.W. and Grime, J.P., 1996. An experimental study of plant community

invasibility. *Ecology*, 77(3), pp.776–790.

Byers, J.E., 2000. Competition between two estuarine snails: implications for invasions of exotic species. *Ecology*, 81(5), pp.1225-1239.

Cardona, L., 2006. Habitat selection by grey mullets (Osteichthyes: Mugilidae) in Mediterranean estuaries: the role of salinity. *Scientia Marina*, 70(3), pp.443–455.

Careau, V., Thomas, D., Humphries, M.M. and Réale, D., 2008. Energy metabolism and animal personality*. Oikos*, 117(5), pp.641-653.

Chabot, D., Steffensen, J.F. and Farrell, A.P., 2016. The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, 88(1), pp.81–121.

Chapin, F.S., Sala, O.E., Burke, I.C., Grime, J.P., Hooper, D.U., Lauenroth, W.K., Lombard, A., Mooney, H.A., Mosier, A.R., Naeem, S. and Pacala, S.W., 1998. Ecosystem consequences of changing biodiversity. *Bioscience,* 48(1), pp.45-52.

Chapman, J.K., Anderson, L.G., Gough, C.L. and Harris, A.R., 2016. Working up an appetite for lionfish: a market-based approach to manage the invasion of Pterois volitans in Belize. *Marine Policy*, 73, pp.256-262.

Christensen, E.A., Grosell, M. and Steffensen, J.F., 2019. Maximum salinity tolerance and osmoregulatory capabilities of European perch Perca fluviatilis populations originating from different salinity habitats. *Conservation physiology*, 7(1), p.coz004.

Clavero, M. and García-Berthou, E., 2005. Invasive species are a leading cause of animal extinctions. *Trends in ecology & evolution*, 20(3), p.110.

Claydon, J.A.B., Calosso, M.C. and Traiger, S.B., 2012. Progression of invasive lionfish in seagrass, mangrove and reef habitats. *Marine Ecology Progress*

Series, 448, pp.119–129.

Cooke, S.J., Hinch, S.G., Farrell, A.P., Patterson, D.A., Miller-Saunders, K., Welch, D.W., Donaldson, M.R., Hanson, K.C., Crossin, G.T., Mathes, M.T. and Lotto, A.G., 2008. Developing a mechanistic understanding of fish migrations by linking telemetry with physiology, behavior, genomics and experimental biology: an interdisciplinary case study on adult Fraser River sockeye salmon. *Fisheries*, 33(7), pp.321-339.

Côté, I.M. and Maljković, A., 2010. Predation rates of Indo-Pacific lionfish on Bahamian coral reefs. *Marine Ecology Progress Series*, 404, pp.219–225.

Côté, I.M. and Smith, N.S., 2018. The lionfish Pterois sp. invasion: Has the worst-case scenario come to pass? *Journal of Fish Biology*, 92(3), pp.660–689.

Côté, I.M., Green, S.J. and Hixon, M.A., 2013. Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. *Biological Conservation*, 164, pp.50–61.

Courtenay, W.R., 1995. Marine fish introductions in South Eastern Florida. *American Fisheries Society Introduced Fish Section Newsletter*, 14(1), pp.2–3.

Crowl, T.A., Crist, T.O., Parmenter, R.R., Belovsky, G. and Lugo, A.E., 2008. The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment*, 6(5), pp.238-246.

Cure, K., Benkwitt, C.E., Kindinger, T.L., Pickering, E.A., Pusack, T.J., McIlwain, J.L. and Hixon, M.A., 2012. Comparative behavior of red lionfish Pterois volitans on native Pacific versus invaded Atlantic coral reefs. *Marine Ecology Progress Series*, 467, pp.181-192.

Cutts, C.J., Metcalfe, N.B. and Taylor, A.C., 2002. Juvenile Atlantic Salmon (Salmo salar) with relatively high standard metabolic rates have small metabolic scopes. *Functional Ecology*, 16(1), pp.73–78.

Daehler, C.C., 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), pp.183–211.

Dahlgren, C. and Marr, J., 2004. Back reef systems: Important but overlooked components of tropical marine ecosystems. *Bulletin of Marine Science*, 75(2), pp.145–152.

Dalziel, A.C., Vines, T.H. and Schulte, P.M., 2012. Reductions in prolonged swimming capacity following freshwater colonization in multiple threespine stickleback populations. *Evolution*, 66(4), pp.1226–1239.

Davidson, A.M., Jennions, M. and Nicotra, A.B., 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology letters*, 14(4), pp.419–431.

Davis, M.A., Grime, J.P. and Thompson, K., 2000. Fluctuating resources in plant communities: a general theory of invisibility. *Journal of ecology*, 88(3), pp.528–534.

Diele, K. and Simith, D.J.B., 2006. Salinity tolerance of northern Brazilian mangrove crab larvae, Ucides cordatus (Ocypodidae): Necessity for larval export? *Estuarine, Coastal and Shelf Science*, 68(3–4), pp.600–608.

Doody, J.S., Mayes, P., Clulow, S., Rhind, D., Green, B., Castellano, C.M., D'Amore, D. and Mchenry, C., 2014. Impacts of the invasive cane toad on aquatic reptiles in a highly modified ecosystem: the importance of replicating impact studies. *Biological invasions*, 16(11), pp.2303-2309.

Dutil, J.D., Lambert, Y., Boucher, E., 1997. Does higher growth rate in Atlantic

cod. Gadus morhua at low salinity result from lower standard metabolic rate or increased protein digestibility? *Can J. Fish. Aquat. Sci*. 54, 99103.

Ege, R. and Krogh, A., 1914. On the relation between the temperature and the respiratory exchange in fishes. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, 7(1), pp.48–55.

Ellis, R. D. and M. E. Faletti., 2016. Native grouper indirectly ameliorates the negative effects of invasive lionfish. *Marine Ecology Progress Series*. 558, pp.267-279.

Ellis, W.L. and Bell, S.S., 2004. Conditional use of mangrove habitats by fishes: depth as a cue to avoid predators. *Estuaries*, 27(6), pp.966–976.

Evangelista, P.H., Young, N.E., Schofield, P.J. and Jarnevich, C.S., 2016. Modeling suitable habitat of invasive red lionfish Pterois volitans (Linnaeus, 1758) in North and South America's coastal waters. *Aquatic Invasions*, 11(3).

Faunce, C. H. and J. E. Serafy., 2006. Mangroves as fish habitat: 50 years of field studies. *Marine Ecology Progress Series* 318:1-18.

Febry, B.Y.R. and Lutz, P., 1987. Activity partitioning in fish: the activity -related cost of osmoregulation in a euryhaline cichlid. *Journal of Experimental Biology*, 85, pp.63–85.

Ferraris, R., Almendras, J. and Jazul, A., 1988. Changes in plasma osmolality and chloride concentration during abrupt transfer of milkfish (Chanos chanos) from seawater to different test salinities. *Aquaculture* 70: 145-157

Ferreira, C.E., Luiz, O.J., Floeter, S.R., Lucena, M.B., Barbosa, M.C., Rocha, C.R. and Rocha, L.A., 2015. First record of invasive lionfish (Pterois volitans) for the Brazilian coast. *PloS one*, 10(4): e0123002.

Fishelson, L., 1997. Experiments and observations on food consumption,

growth and starvation in Dendrochirus brachypterus and Pterois volitans (Pteroinae, Scorpaenidae). *Environmental Biology of Fishes*, 50(4), pp.391–403.

Forrester, G.E. and Steele, M.A., 2004. Predators, prey refuges, and the spatial scaling of density-dependent prey mortality. *Ecology*, 85(5), pp.1332–1342.

Freeland, W.J. and Martin, K.C., 1985. The rate of range expansion by Bufo marinus in Northern Australia, 1980-84. *Wildlife Research*, 12(3), pp.555–559.

Fu, S.J., Xie, X.J. and Cao, Z.D., 2005. Effect of meal size on postprandial metabolic response in southern catfish (Silurus meridionalis). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 140(4), pp.445–451.

Gallagher, S.E., 2013. Establishing a culinary market for lionfish species through a market-based organization to mitigate the environmental impacts of the invasive species (*Doctoral dissertation, College of Charleston*).

Galil, B.S., 2007. Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. *Marine Pollution Bulletin*, 55(7–9), pp.314–322.

Gaumet, F., Boeuf, G., Severe, A., Le Roux, A. and Mayer-Gostan, N., 1995. Effects of salinity on the ionic balance and growth of juvenile turbot. J*. Fish Biol.* 47, 865876.

Gilman, E.L., Ellison, J., Duke, N.C. and Field, C., 2008. Threats to mangroves from climate change and adaptation options: a review. *Aquatic botany*, 89(2), pp.237-250.

Giuliani, G., De Bono, A., Kluser, S., and Peduzzi, P. (2004). Overfishing, a major threat to the global marine ecology.

Golani, D. and Sonin, O., 1992. New records of the Red Sea fishes, Pterois

miles (Scorpaenidae) and Pteragogus pelycus (Labridae) from the eastern Mediterranean Sea. *Japanese Journal of Ichthyology*, 39 (2), 167-169

Goodwin, B.J., McAllister, A.J. and Fahrig, L., 1999. Predicting invasiveness of plant species based on biological information. *Conservation biology*, 13(2), pp.422–426.

Green, S.J., Akins, J.L., Maljković, A. and Côté, I.M., 2012. Invasive lionfish drive Atlantic coral reef fish declines. *PloS one*, 7(3): e32596.

Green, S.J., Dilley, E.R., Benkwitt, C.E., Davis, A.C., Ingeman, K.E., Kindinger, T.L., Tuttle, L.J. and Hixon, M.A., 2019. Trait-mediated foraging drives patterns of selective predation by native and invasive coral-reef fishes. *Ecosphere*, 10(6), p.e02752.

Greenwood, A.K., Wark, A.R., Fernald, R.D. and Hofmann, H.A., 2008. Expression of arginine vasotocin in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. *Proceedings of the Royal Society B: Biological Sciences*, 275(1649), pp.2393-2402.

Grieve, B.D., Curchitser, E.N. and Rykaczewski, R.R., 2016. Range expansion of the invasive lionfish in the Northwest Atlantic with climate change. *Marine Ecology Progress Series*, 546, pp.225–237.

Grøtan, K., Østbye, K., Taugbøl, A. and Vøllestad, L.A., 2012. No short-term effect of salinity on oxygen consumption in threespine stickleback (Gasterosteus aculeatus) from fresh, brackish, and salt water. *Canadian journal of zoology*, 90(12), pp.1386-1393.

Hackerott, S., A. Valdivia, S. J. Green, I. M. Côté, C. E. Cox, L. Akins, C. A. Layman, W. F. Precht, and J. F. Bruno. 2013. Native predators do not influence invasion success of Pacific lionfish on Caribbean reefs. *PLoS*

ONE 8:e68259. doi:68210.61371/journal.pone.0068259.

Hackerott, S., Valdivia, A., Cox, C.E., Silbiger, N.J. and Bruno, J.F., 2017. Invasive lionfish had no measurable effect on prey fish community structure across the Belizean Barrier Reef. *PeerJ*, 5: e3270.

Hare, J.A. and Whitfield, P.E., 2003. An integrated assessment of the introduction of lionfish (Pterois volitans/miles complex) to the western Atlantic Ocean.

Hixon, M., 1991. Tropical and temperate reef fishes comparison of community structures. *The ecology of fishes on coral reefs*, pp.509–563.

Hodges, K.E., 2008. Defining the problem: terminology and progress in ecology. *Frontiers in Ecology and the Environment*, 6(1), pp.35–42.

Hussey, N.E., Kessel, S.T., Aarestrup, K., Cooke, S.J., Cowley, P.D., Fisk, A.T., Harcourt, R.G., Holland, K.N., Iverson, S.J., Kocik, J.F. and Flemming, J.E.M., 2015. Aquatic animal telemetry: a panoramic window into the underwater world. *Science*, 348(6240), p.1255642.

Huth, W.L., McEvoy, D.M. and Morgan, O.A., 2016. Controlling an invasive species through consumption: private and public values of eating lionfish. *Working Paper-Department of Economics, Appalachian State University*, (16-05).

Imsland, A.K., Foss, A., Gunnarsson, S. et al., 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot. Scophthalmus maximus . *Aquaculture* 198, 353367.

Ingeman, K.E., Albins, M.A., Benkwitt, C.E., Green, S.J., Kindinger, T.L., Tuttle, L.J. and Hixon, M.A., 2017. Resolving differences in observed impacts of invasive lionfish and clarifying advice to managers. *PeerJ Preprints*, 5, p.e3455v1.

Intergovernmental Panel on Climate Change., 2014. Mitigation of climate change. *Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, 1454.

International Union for Conservation of Nature, 2000. IUCN Guidelines for the Prevention of Biodiversity Loss Caused by Alien Invasive Species

Jensen, F., Lecklin, T., Busk, M., Bury, N., Wilson, R., Wood, C. and Grosell, M. 2002. Physiological impact of salinity increase at organism and red blood cell levels in the European flounder (Platichthys flesus). *J. Exp. Mar. Biol. Ecol.* 274: 159–174

Jud, Z.R. and Layman, C.A., 2012. Site fidelity and movement patterns of invasive lionfish, Pterois spp., in a Florida estuary. *Journal of Experimental Marine Biology and Ecology*, 414, pp.69–74.

Jud, Z.R., Layman, C.A., Lee, J.A. and Arrington, D.A., 2011. Recent invasion of a Florida (USA) estuarine system by lionfish Pterois volitans/P. miles. *Aquatic Biology*, 13(1), pp.21-26.

Jud, Z.R., Nichols, P.K. and Layman, C.A., 2015. Broad salinity tolerance in the invasive lionfish Pterois spp. may facilitate estuarine colonization. *Environmental Biology of Fishes*, 98(1), pp.135–143.

Juliano, S.A. and Philip Lounibos, L., 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology letters*, 8(5), pp.558–574.

Killen, S.S., Atkinson, D. and Glazier, D.S., 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters*, 13(2), pp.184–193.

Kulbicki, M., Beets, J., Chabanet, P., Cure, K., Darling, E., Floeter, S.R., Galzin, R., Green, A., Harmelin-Vivien, M., Hixon, M. and Letourneur, Y., 2012.

Distributions of Indo-Pacific lionfishes Pterois spp. in their native ranges: implications for the Atlantic invasion. *Marine Ecology Progress Series*, 446, pp.189-205.

Laegdsgaard, P. and Johnson, C., 2001. Why do juvenile fish utilise mangrove habitats? *Journal of experimental marine biology and ecology*, 257(2), pp.229–253.

Lambert, Y., Dutil, J.D. and Munro, J., 1994. Effect of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod Gadus morhua.. *Can. J. Fish. Aquat. Sci*. 51, 15691576.

Layman, C.A. and Allgeier, J.E., 2012. Characterizing trophic ecology of generalist consumers: a case study of the invasive lionfish in The Bahamas. *Marine Ecology Progress Series*, 448, pp.131–141.

Lönnstedt, O.M., Ferrari, M.C.O. and Chivers, D.P., 2014. Lionfish predators use flared fin displays to initiate cooperative hunting. *Biology letters*, 10(6), p.20140281.

Luo, J., Serafy, J.E., Sponaugle, S., Teare, P.B. and Kieckbusch, D., 2009. Movement of gray snapper Lutjanus griseus among subtropical seagrass, mangrove, and coral reef habitats. *Marine Ecology Progress Series*, 380, pp.255-269.

MacDonald, J.A., Shahrestani, S. and Weis, J.S., 2009. Behavior and space utilization of two common fishes within Caribbean mangroves: implications for the protective function of mangrove habitats. *Estuarine, Coastal and Shelf Science*, 84(2), pp.195–201.

Mack, R.N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M. and Bazzaz, F.A., 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological applications*, 10(3), pp.689-710.

Martins, C.I., Castanheira, M.F., Engrola, S., Costas, B. and Conceição, L.E., 2011. Individual differences in metabolism predict coping styles in fish. *Applied Animal Behaviour Science*, 130(3-4), pp.135-143.

Mateo, I., Durbin, E.G., Appeldoorn, R.S., Adams, A.J., Juanes, F., Kingsley, R., Swart, P. and Durant, D., 2010. Role of mangroves as nurseries for French grunt Haemulon flavolineatum and schoolmaster Lutjanus apodus assessed by otolith elemental fingerprints. *Marine Ecology Progress Series*, 402, pp.197-212.

McCarthy, I.D., 2000. Temporal repeatability of relative standard metabolic rate in juvenile Atlantic salmon and its relation to life history variation. *Journal of Fish Biology*, 57(1), pp.224–238.

McCormick, M.I. and Allan, B.J., 2016. Lionfish misidentification circumvents an optimized escape response by prey. *Conservation physiology*, 4(1).

McGaw, I.J. and Van Leeuwen, T.E., 2017. Metabolic costs of the mechanical components of the apparent specific dynamic action in the Dungeness crab, Cancer magister. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*, 210(May), pp.22–27.

McGaw, I.J., 2006. Feeding and digestion in low salinity in an osmoconforming crab, Cancer gracilis II. Gastric evacuation and motility. *Journal of experimental biology*, 209(19), pp.3777–3785.

Molnar, J.L., Gamboa, R.L., Revenga, C. and Spalding, M.D., 2008. Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, 6(9), pp.485-492.

Morgan, J.D. and Iwama, G.K., 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (Oncorhynchus mykiss) and fall chinook salmon (Oncorhynchus tshawytscha). Canadian

Journal of Fisheries and Aquatic Sciences, 48(11), pp.2083–2094.

Morris, J.A. and Akins, J.L., 2009. Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. *Environmental Biology of Fishes*, 86(3), pp.389–398.

Morris, J.A.Jr. and Whitfield, P.E., 2009. Biology, ecology, control and management of the Invasive Indo-Pacific Lionfish: An updated integrated assessment. *NOAA Technical Memorandum*, NOS NCCOS 99, 57 pp.

Mumby, P.J., Edwards, A.J., Arias-González, J.E., Lindeman, K.C., Blackwell, P.G., Gall, A., Gorczynska, M.I., Harborne, A.R., Pescod, C.L., Renken, H. and Wabnitz, C.C., 2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. *Nature*, 427(6974), p.533.

Mumby, P.J., Harborne, A.R. and Brumbaugh, D.R., 2011. Grouper as a natural biocontrol of invasive lionfish. *PloS one*, 6(6): e21510.

Nagelkerken, I., Kleijnen, S., Klop, T., Van Den Brand, R.A.C.J., de La Moriniere, E.C. and Van der Velde, G., 2001. Dependence of Caribbean reef fishes on mangroves and seagrass beds as nursery habitats: a comparison of fish faunas between bays with and without mangroves/seagrass beds. *Marine Ecology Progress Series*, 214, pp.225-235.

Nagelkerken, I.S.J.M., Blaber, S.J.M., Bouillon, S., Green, P., Haywood, M., Kirton, L.G., Meynecke, J.O., Pawlik, J., Penrose, H.M., Sasekumar, A. and Somerfield, P.J., 2008. The habitat function of mangroves for terrestrial and marine fauna: a review. *Aquatic botany*, 89(2), pp.155-185.

National Research Council, 2010. Guide for the care and use of laboratory animals, *National Academies Press*.

Nonnotte, G. and Truchot, D., 1990. Time course of extracellular acid-base

adjustments under hypo- or hyperosmotic conditions in the euryhaline fish Platichthys ftesus *J. Fish Biol*. 36: 181-190

Norin, T., Malte, H. and Clark, T.D., 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *Journal of Experimental Biology*, 217(2), pp.244–251.

Peake, J., Bogdanoff, A.K., Layman, C.A., Castillo, B., Reale-Munroe, K., Chapman, J., Dahl, K., Patterson III, W.F., Eddy, C., Ellis, R.D. and Faletti, M., 2018. Feeding ecology of invasive lionfish (Pterois volitans and Pterois miles) in the temperate and tropical western Atlantic. *Biological Invasions*, 20(9), pp.2567-2597.

Pejchar, L. and Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. *Trends in ecology & evolution*, 24(9), pp.497–504.

Penney, C.M., Patton, R.L., Whiteley, N.M., Driedzic, W.R. and McGaw, I.J., 2016. Physiological responses to digestion in low salinity in the crabs Carcinus maenas and Cancer irroratus. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 191, pp.127-139.

Pimiento, C., Nifong, J.C., Hunter, M.E., Monaco, E. and Silliman, B.R., 2015. Habitat use patterns of the invasive red lionfish Pterois volitans: a comparison between mangrove and reef systems in San Salvador, Bahamas. *Marine Ecology*, 36(1), pp.28-37.

Prakash, S., Balamurugan, J., Kumar, T.A. and Balasubramanian, T., 2012. Invasion and abundance of reef inhabiting fishes in the Vellar estuary, southeast coast of India, especially the lionfish Pterois volitans Linnaeus. *Current Science*, pp.941-944.

Quiñones, R.M. and Mulligan, T.J., 2005. Habitat use by juvenile salmonids in the Smith River estuary, California. *Transactions of the American Fisheries*

Society, 134(5), pp.1147–1158.

Radford, I.J. and Cousens, R.D., 2000. Invasiveness and comparative life-history traits of exotic and indigenous Senecio species in Australia. *Oecologia,* 125(4), pp.531–542.

Randall, J., 2005. Reef and shore fishes of the South Pacific: New Caledonia to Tahiti and the Pitcairn Islands. *University of Hawaii Press*, Honolulu, H

Raymond, W.W., Albins, M.A. and Pusack, T.J., 2015. Competitive interactions for shelter between invasive Pacific red lionfish and native Nassau grouper. *Environmental biology of fishes*, 98(1), pp.57–65.

Reidy, S.P., Nelson, J.A., Tang, Y. and Kerr, S.R., 1995. Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. *Journal of Fish Biology*, 47(3), pp.377-386.

Richardson, D.M. and Ricciardi, A., 2013. Misleading criticisms of invasion science: a field guide. *Diversity and Distributions*, 19(12), pp.1461–1467.

Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Bd. Can*., 191, pp.1-382.

Robertson, A.I. and Duke, N.C., 1987. Mangroves as nursery sites: comparisons of the abundance and species composition of fish and crustaceans in mangroves and other nearshore habitats in tropical Australia. *Marine Biology*, 96(2), pp.193–205.

RStudio Team (2019). RStudio: Integrated Development for R. *RStudio, Inc*., Boston, MA URL http://www.rstudio.com/.

Ruiz, G.M., Fofonoff, P.W., Steves, B.P. and Carlton, J.T., 2015. Invasion history and vector dynamics in coastal marine ecosystems: a North American perspective. *Aquatic ecosystem health & management*, 18(3), pp.299-311.

Rupia, E.J., Binning, S.A., Roche, D.G. and Lu, W., 2016. Fight-flight or freeze-hide? Personality and metabolic phenotype mediate physiological defence responses in flatfish. *Journal of Animal Ecology*, 85(4), pp.927-937.

Sabath, M.D., Boughton, W.C. and Easteal, S., 1981. Expansion of the range of the introduced toad Bufo marinus in Australia from 1935 to 1974. *Copeia,* pp.676–680.

Sampaio, L. and Bianchini, A., 2002. Salinity effects on osmoregulation and growth of the euryhaline flounder Paralichthys orbignyanus. *J. Exp. Mar. Biol. Ecol.* 269: 187– 196

Sandilyan, S. and Kathiresan, K., 2015. Mangroves as bioshield: an undisputable fact. *Ocean & Coastal Management*, 103, pp.94–96.

Santos, G.A., Schrama, J.W., Mamauag, R.E.P., Rombout, J.H.W.M. and Verreth, J.A.J., 2010. Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (Dicentrarchus labrax): the combined effects of fish crowding and water quality deterioration. *Aquaculture*, 299(1-4), pp.73-80.

Satheeshkumar, P., Manjusha, U., Pillai, N.G.K. and Senthil Kumar, D., 2012. Puducherry mangroves under sewage pollution threat need conservation. *Current Science*, 102(1), pp.13-14.

Schofield, P.J., 2009. Geographic extent and chronology of the invasion of non-native lionfish (Pterois volitans [Linnaeus 1758] and P. miles [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. *Aquatic Invasions*, 4(3), pp.473–479.

Schreck, C.B., Olla, B.L. and Davis, M.W., 1997. Behavioral responses to stress. *Fish stress and health in aquaculture*, 62, pp.145-170.

Schultz, E.T., 1986. Pterois volitans and Pterois miles: two valid species.

Copeia 1986(3):686–690

Secor, S.M. and Faulkner, A.C., 2002. Effects of meal size, meal type, body temperature, and body size on the specific dynamic action of the marine toad, Bufo marinus. *Physiological and Biochemical Zoology*, 75(6), pp.557–571.

Secor, S.M., 2009. Specific dynamic action: a review of the postprandial metabolic response. *Journal of Comparative Physiology B*, 179(1), pp.1–56.

Serafy, J.E., Shideler, G.S., Araújo, R.J. and Nagelkerken, I., 2015. Mangroves enhance reef fish abundance at the Caribbean regional scale. *PloS one*, 10(11): e0142022.

Serrano, X.M., 2008, Ecophysiology of the Gray Snapper (Lutjanus griseus): Salinity Effects on Abundance, Physiology and Behavior. *Open Access Theses*. 144

Smith, N.S., Green, S.J., Akins, J.L., Miller, S. and Côté, I.M., 2017. Density-dependent colonization and natural disturbance limit the effectiveness of invasive lionfish culling efforts. *Biological Invasions*, 19(8), pp.2385-2399.

Snyder, D.B. and Burgess, G.H., 2007. The Indo-Pacific red lionfish, Pterois volitans (Pisces: Scorpaenidae), new to Bahamian ichthyofauna. *Coral Reefs*, 26(1), p.175.

Soofiani, N.M. and Hawkins, A.D., 1982. Energetic costs at different levels of feeding in juvenile cod, Gadus morhua L. *Journal of Fish Biology*, 21(5), pp.577–592.

Steffensen, J.F., 1989. Some errors in respirometry of aquatic breathers: How to avoid and correct for them. *Fish Physiology and Biochemistry*, 6(1), pp.49–59.

Stevens, D.E. and Dizon, A.E., 1982. Energetics of Locomotion in Warm-Bodied Fish. *Annual Review of Physiology*, 44(1), pp.121–131.

Sutherland, W.J., Clout, M., Côté, I.M., Daszak, P., Depledge, M.H., et al., 2010. A horizon scan of global conservation issues for 2010. *Trends in Ecology & Evolution* 25: 1–7.

Thresher, R.E., 1999. Diversity, Impacts and Options for Managing Invasive Marine Species in Australian Waters. *Australasian Journal of Environmental Management*, 6(3), pp.137–148.

Torchin, M.E., Lafferty, K.D. and Kuris, A.M., 2001. Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions*, 3(4), pp.333-345.

Torchin, M.E., Byers, J.E. and Huspeni, T.C., 2005. Differential parasitism of native and introduced snails: replacement of a parasite fauna. *Biological Invasions*, 7(6), pp.885-894.

Turan, C., Ergüden, D., Gürlek, M., Yağlıoğlu, D., Uyan, A. and Uygur, N., 2014. First record of the Indo-Pacific lionfish Pterois miles (Bennett, 1828) (Osteichthyes: Scorpaenidae) for the Turkish marine waters. *Journal of the Black Sea/Mediterranean Environment*, 20(2).

Tuttle, L.J., Sikkel, P.C., Cure, K. and Hixon, M.A., 2017. Parasite-mediated enemy release and low biotic resistance may facilitate invasion of Atlantic coral reefs by Pacific red lionfish (Pterois volitans). *Biological invasions*, 19(2), pp.563-575.

Van Clef, M. and Stiles, E.W., 2001. Seed longevity in three pairs of native and non-native congeners: assessing invasive potential. *Northeastern Naturalist*, 8(3), pp.301–311.

Van Leeuwen, T.E., Rosenfeld, J.S. and Richards, J.G., 2012. Effects of food

ration on SMR: Influence of food consumption on individual variation in metabolic rate in juvenile coho salmon (Onchorhynchus kisutch). *Journal of Animal Ecology*, 81(2), pp.395–402.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L. and Westbrooks, R., 1996. Biological invasions as global environmental change. *American Scientist*, 84 (1996), pp. 468-478

Webb, P.W., 1975. Hydrodynamics and energetics of fish propulsion. *Bulletin of the fisheries research board of Canada*, 190, pp.1–159.

Wells, S. and Ravilious, C., 2006. *In the front line: shoreline protection and other ecosystem services from mangroves and coral reefs* (No. 24). United Nations Environment Programme World Conservation Monitoring Centre, Cambridge, UK

Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiological reviews*, 77(3), pp.591-625.

Whitfield, P.E. and Hare, J. A., 2003. An Integrated Assessment of the Introduction of Lionfish (Pterois volitans / miles complex) to the Western Atlantic Ocean. *NOAA Technical Memorandum*, p.21.

Whitfield, P.E., Hare, J.A., David, A.W., Harter, S.L., Munoz, R.C. and Addison, C.M., 2007. Abundance estimates of the Indo-Pacific lionfish Pterois volitans/miles complex in the Western North Atlantic. *Biological Invasions*, 9(1), pp.53-64.

Wilcove, D.S. and Chen, L.Y., 1998. Management costs for endangered species. *Conservation Biology*, 12(6), pp.1405–1407.

Wilcox, C.L., Motomura, H., Matsunuma, M. and Bowen, B.W., 2017. Phylogeography of lionfishes (Pterois) indicate taxonomic over splitting and hybrid origin of the invasive Pterois volitans. *Journal of Heredity*, 109(2),

pp.162-175.

Wolff, M., Koch, V. and Isaac, V., 2000. A trophic flow model of the Caeté mangrove estuary (North Brazil) with considerations for the sustainable use of its resources. Estuarine, *Coastal and Shelf Science*, 50(6), pp.789–803.